The Patent Office

For Innovation

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed A R Buckell

Dated 11 October 2006

CERTIFIED COPY OF
PRIORITY DOCUMENT

...ents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
04 AUG 2003
RECEIVED BY HAND

The Patent Office
PATENTS • DESIGNS • COPYRIGHT • TRADE MARKS

05AUG03 E827646-1 002246
P01/7700 0.00-0318213.6

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

P01534-GBR CLM

2. Patent application number
(The Patent Office will fill in this part)

04 AUG 2003

0318213.6

Full name, address and postcode of the or of each applicant *(underline all surnames)*

OXFORD BIOMEDICA (UK) LIMITED
Medawar Centre
Robert Robinson Avenue
The Oxford Science Park
Oxford OX4 4GA

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

GB

76671570001

4. Title of the invention

VECTOR SYSTEM

5. Name of your agent *(if you have one)*

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

21 New Fetter Lane
London
EC4A 1DA

Patents ADP number *(if you know it)*

59006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
GB	0223076.1	04 10 02
GB	0228314.1	04 12 02

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	60
Claim(s)	16
Abstract	1
Drawing(s)	9 +9

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature D Young & Co. Date 04 August 2003

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Catherine Mallalieu — 020 7353 4343

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

VECTOR SYSTEM

FIELD OF THE INVENTION

The present invention relates to a vector system. In particular, the present invention relates to a vector system capable of delivering an entity of interest ("EOI") – such as a nucleotide sequence of interest ("NOI") - to a target site, such as for the treatment of diseases affecting the central nervous system (CNS).

In one preferred aspect, the present invention relates to a viral vector system capable of delivering a nucleotide sequence of interest ("NOI") to a target site.

In a more preferred aspect, the present invention relates to a lentiviral vector system capable of delivering a nucleotide sequence of interest ("NOI") to a target site.

More in particular, the present invention relates to a retroviral vector useful in gene therapy.

BACKGROUND TO THE INVENTION

Gene therapy includes any one or more of: the addition, the replacement, the deletion, the supplementation, the manipulation etc. of one or more nucleotide sequences in, for example, one or more targetted sites - such as targetted cells. If the targetted sites are targetted cells, then the cells may be part of a tissue or an organ. General teachings on gene therapy may be found in Molecular Biology (Ed Robert Meyers, Pub VCH, such as pages 556-558).

By way of further example, gene therapy also provides a means by which any one or more of: a nucleotide sequence, such as a gene, can be applied to replace or supplement a defective gene; a pathogenic gene or gene product can be eliminated; a new gene can be added in order, for example, to create a more favourable phenotype; cells can be manipulated at the molecular level to treat cancer (Schmidt-Wolf and Schmidt-Wolf,

1994, Annals of Hematology 69;273-279) or other conditions - such as immune, cardiovascular, neurological, inflammatory or infectious disorders; antigens can be manipulated and/or introduced to elicit an immune response - such as genetic vaccination.

In recent years, retroviruses have been proposed for use in gene therapy. Essentially, retroviruses are RNA viruses with a life cycle different to that of lytic viruses. In this regard, when a retrovirus infects a cell, its genome is converted to a DNA form. In otherwords, a retrovirus is an infectious entity that replicates through a DNA intermediate. More details on retroviral infection etc. are presented later on.

With regard to the genetic structure of a viral vector, the gene *env* encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction leads ultimately to fusion of the viral membrane with the cell membrane.

Although uncleaved Env proteins are able to bind to the receptor, the cleavage event itself is necessary to activate the fusion potential of the protein, which is necessary for entry of the virus into the host cell. Typically, both SU and TM proteins are glycosylated at multiple sites. However, in some viruses, exemplified by MLV, TM is not glycosylated.

Although the SU and TM proteins are not always required for the assembly of enveloped virion particles as such, they do play an essential role in the entry process. In this regard, the SU domain binds to a receptor molecule - often a specific receptor molecule - on the target cell. It is believed that this binding event activates the membrane fusion-inducing potential of the TM protein after which the viral and cell membranes fuse. In some viruses, notably MLV, a cleavage event - resulting in the removal of a short portion of the cytoplasmic tail of TM - is thought to play a key role in uncovering the full fusion activity of the protein (Brody *et al* 1994 J. Virol. 68: 4620-4627, Rein *et al* 1994 J. Virol. 68: 1773-1781). This cytoplasmic "tail", distal to the membrane-spanning segment of

TM remains on the internal side of the viral membrane and it varies considerably in length in different retroviruses.

Thus, the specificity of the SU/receptor interaction can define the host range and tissue tropism of a retrovirus. In some cases, this specificity may restrict the transduction potential of a recombinant retroviral vector. For this reason, many gene therapy experiments have used MLV. A particular MLV that has an envelope protein called 4070A is known as an amphotropic virus, and this can also infect human cells because its envelope protein "docks" with a phosphate transport protein that is conserved between man and mouse. This transporter is ubiquitous and so these viruses are capable of infecting many cell types. In some cases however, it may be beneficial, especially from a safety point of view, to specifically target restricted cells. To this end, several groups have engineered a mouse ecotropic retrovirus, which unlike its amphotropic relative normally only infects mouse cells, to specifically infect particular human cells. Replacement of a fragment of an envelope protein with an erythropoietin segement produced a recombinant retrovirus which then bound specifically to human cells that expressed the erythropoietin receptor on their surface, such as red blood cell precursors (Maulik and Patel 1997 "Molecular Biotechnology: Therapeutic Applications and Strategies" 1997. Wiley-Liss Inc. pp 45.).

Replacement of the *env* gene with a heterologous *env* gene is an example of a technique or strategy called pseudotyping. Pseudotyping can confer one or more advantages. For example, with the lentiviral vectors, the *env* gene product of the HIV based vectors would restrict these vectors to infecting only cells that express a protein called CD4. But if the *env* gene in these vectors has been substituted with *env* sequences from other RNA viruses, then they may have a broader infectious spectrum (Verma and Somia 1997 Nature 389:239-242).

More generally, delivery of therapeutic molecules to the CNS represents an important challenge for the treatment of neurodegenerative diseases. Limitations to overcome

include (i) the presence of the blood-brain barrier, (ii) side effects associated with systemic administration, and (iii) instability of the molecules.

One problem with gene therapy approaches in the treatment of, for example, Parkinson's disease, is that brain is a difficult and complex organ to target (Raymon H.K. *et al* (1997) Exp. Neur. 144: 82-91). The usual route is by injection of vectors to the striatum (Bilang-Bleuel et al (1997) Proc. Acad. Natl. Sci. USA 94:8818-8823; Choi-Lundberg et al (1998) Exp. Neurol.154:261-275) or to near the substantia nigra (Choi-Lundberg et al (1997) Science 275:838-841; Mandel et al (1997)) Proc. Acad. Natl. Sci. USA 94:14083-14088). It is technically difficult to inject directly into the some parts of the brain, for example because of their location and/or size. The substantia nigra lies deep in the brain and direct injection to this area can cause lesion of axons, resulting in damage. The striatum, (in particular the caudate putamen) is a relatively easy target because it is larger and more dorsal than the substantia nigra. It has been used extensively for transplantation in Parkinson's disease, and there is currently thought to be less than 1% risk involved in the operation. Similar problems exist in relation to other parts of the CNS.

Hence, it is desirable to find a mechanism for transducing parts of the brain and other parts of the CNS which are difficult to reach by direct injection. It is also desirable to find an administration strategy for cranial gene therapy which minimises the number and complexity of brain injections. It is also desirable to achieve good penetration and distribution throughout the nervous system following administration.

It has been thought that pseudotyping might alleviate some of the above-mentioned problems. However, the transduction and expression characteristics of pseudotyped vectors have not yet been fully determined and there remains the need to provide further and improved vectors.

By way of example, Mazarakis et al (2001) Human Molecular Genetics 10(19):2109-2121 teaches that a lentiviral vector pseudotyped with VSV G transduced muscle cells

surrounding an injection site, but did not result in expression in any cells in the spinal cord.

WO02/36170 teaches the use of a wild type rabies G protein to achieve retrograde transport, and particularly transduction of a TH positive neuron. We have found that it is possible to achieve good biodistribution of an entity of interest (EOI) through a mechanism other than retrograde transport using both rabies G and using the envelope protein from CVS (ChaliengeVirus Standard). Thus, it will be appreciated that this enables sites to be targeted through administration sites other than those which would be available using the retrograde transport mechanism. Whilst not wishing to be bound by any theory we believe that this high level of distribution may be achieved through a diffusion mechanism. In contrast, we have found that VSV G pseudotyping does not give rise to such biodistribution confirming the surprising result demonstrated herein. It will be appreciated that good biodistribution is important so that different parts of the central nervous system can be accessed through a localised site of administration. This particularly helps where penetration by an EOI to sites which are not readily accessible is required. We have also found that pseudotyped EIAV vectors give a particularly good effect.

We have also found that retrograde transport and transduction of cells of the CNS can be achieved using the envelope protein from CVS (Challenge Virus Standard). We believe that we are he first to demonstrate the advantages of lentiviral pseudotyping with a CVS protein.

In addition, we have found that pseudotyping with rabies G and CVS envelope proteins give particular advantages when administered in utero or to a neonate. In these circumstances we have found that one can achieve good transduction in muscle cells, which is surprising give that transduction is poor in adult cells. We have also found that transport, e.g. by retrograde transport, to motor and sensory neurons is enhanced. These results are particularly advantageous where therapy needs to be administered in the early stages of life, e.g. in the case of spinal muscular atropy.

STATEMENTS OF THE PRESENT INVENTION

In a broad aspect, the present invention relates to a vector system that is capable of causing retrograde transport of an entity of interest ("EOI").

As used herein the term "vector system" includes any vector that is capable of infecting or transducing or transforming or modifying a recipient cell with an EOI.

The EOI may be a chemical compound, a biological compound or combinations thereof. By way of example, the EOI may be a protein (such as a growth factor), a nucleotide sequence, an organic and/or an inorganic pharmaceutical (such as an analgesic, an anti-inflammatory, a hormone, a lipid), or combinations thereof.

The vector system of the present invention is capable of delivering the EOI to a site, wherein at that site the EOI may then be distributed and/or penetrate distant sites, e.g. through diffusion or retrograde transport.

Typically the vector system will also comprise an EOI.

According to one aspect of the present invention there is provided use of a vector system to transduce a target site, wherein the vector system travels to the target site by diffusion, and wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof, and further wherein the target site is at least part of the central nervous system.

According to another aspect of the present invention there is provided use of a vector system comprising an EOI to biodistribute the EOI, wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or

fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

According to yet another aspect of the present invention there is provided use of a vector system to transduce a target site, wherein the vector system travels to the target site by retrograde transport, and wherein the vector system is or comprises at least part of a CVS envelope protein or a mutant, variant, homologue or fragment thereof, and further wherein the target site is at least part of the central nervous system.

According to a further aspect of the present invention there is provided use of a vector system to transduce an in utero target site or a target site in a neonate, wherein wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

The vector system can be a non-viral system or a viral system, or combinations thereof. In addition, the vector system itself can be delivered by viral or non-viral techniques.

Viral vector or viral delivery systems include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectors, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery or non-viral vector systems include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof.

In non-viral vector systems of the present invention, the at least part of the rabies G protein (or a mutant, variant, homologue or fragment thereof), and/or the at least part of the CVS protein (or a mutant, variant, homologue or fragment thereof) may be used to encapulate or enshroud an EOI. Thus, for some embodiments, the at least part of the rabies G protein (or a mutant, variant, homologue or fragment thereof), or the at least part of the CVS protein (or a mutant, variant, homologue or fragment thereof) may form a

matrix around the EOI. Here, the matrix may contain other components – such as a liposome type entity.

In some preferred aspects, the vector system is a viral vector system.

In some further preferred aspects, the vector system is a retroviral vector system.

It has also been found that a particular type of vector system – such as viral vector system, preferably a retroviral vector system – according to the present invention is capable of transducing one or more sites which are distant from the site of administration due to retrograde transport of the vector system.

Administration to a single target site may cause transduction of a plurality of target sites. The vector system may travel to the or each target by retrograde transport, optionally in combination with anterograde transport.

In further broad aspects, the present invention relates to:

(i) a method of treating and/or preventing a diseases using such a vector system;

(ii) the use of such a vector system in the manufacture of a pharmaceutical composition to treat and/or prevent a disease;

(iii) a method for analysing the effect of a protein of interest in a cell using such a vector system;

(iv) a method for analysing the function of a gene or protein using such a vector system;

(v) a cell tranduced with such a vector system;

(vi) an immortalised cell made by transduction with such a vector system;

(vii) the use of such an immortalised cell in the manufacture of a medicament; and

(viii) a transplantation method using such an immortalised cell.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a new use of a vector system.

The vector system can be a non-viral system or a viral system.

In some preferred aspects, the vector system is a viral vector system.

In some further preferred aspects, the vector system is a retroviral vector system.

RETROVIRUSES

The concept of using viral vectors for gene therapy is well known (Verma and Somia (1997) Nature 389:239-242).

There are many retroviruses. For the present application, the term "retrovirus" includes: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV) and all other retroviridiae including lentiviruses.

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

In a preferred embodiment, the retroviral vector system is derivable from a lentivirus. Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells (Lewis et al (1992) EMBO J. 3053-3058).

The lentivirus group can be split into "primate" and "non-primate". Examples of primate lentiviruses include the human immunodeficiency virus (HIV), the causative agent of human acquired immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

Details on the genomic structure of some lentiviruses may be found in the art. By way of example, details on HIV and EIAV may be found from the NCBI Genbank database (i.e. Genome Accession Nos. AF033819 and AF033820 respectively). Details of HIV variants may also be found at http://hiv-web.lanl.gov. Details of EIAV variants may be found through http://www.ncbi.nlm.nih.gov.

During the process of infection, a retrovirus initially attaches to a specific cell surface receptor. On entry into the susceptible host cell, the retroviral RNA genome is then copied to DNA by the virally encoded reverse transcriptase which is carried inside the parent virus. This DNA is transported to the host cell nucleus where it subsequently integrates into the host genome. At this stage, it is typically referred to as the provirus. The provirus is stable in the host chromosome during cell division and is transcribed like other cellular genes. The provirus encodes the proteins and other factors required to make more virus, which can leave the cell by a process sometimes called "budding".

Each retroviral genome comprises genes called *gag*, *pol* and *env* which code for virion proteins and enzymes. These genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration, and transcription. They also serve as enhancer-promoter sequences. In other words, the LTRs can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a *psi* sequence located at the 5' end of the viral genome.

The LTRs themselves are identical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5'end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For the viral genome, the site of transcription initiation is at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses have any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: *tat*, *rev*, *tax* and *rex*.

With regard to the structural genes *gag, pol* and *env* themselves, *gag* encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The *pol* gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The *env* gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction leads ultimately to infection by fusion of the viral membrane with the cell membrane.

Retroviruses may also contain "additional" genes which code for proteins other than gag, pol and env. Examples of additional genes include in HIV, one or more of *vif, vpr, vpx, vpu, tat, rev* and *nef*. EIAV has (amongst others) the additional gene *S2*.

Proteins encoded by additional genes serve various functions, some of which may be duplicative of a function provided by a cellular protein. In EIAV, for example, *tat* acts as a transcriptional activator of the viral LTR. It binds to a stable, stem-loop RNA secondary structure referred to as TAR. Rev regulates and co-ordinates the expression of viral genes

through rev-response elements (RRE). The mechanisms of action of these two proteins are thought to be broadly similar to the analogous mechanisms in the primate viruses. The function of S2 is unknown. In addition, an EIAV protein, Ttm, has been identified that is encoded by the first exon of *tat* spliced to the *env* coding sequence at the start of the transmembrane protein.

VECTOR SYSTEMS

The vector system can be a non-viral system or a viral system.

In some preferred aspects, the vector system is a viral vector system.

In some further preferred aspects, the vector system is a retroviral vector system.

The vector system can be used to transfer an EOI to one or more sites of interest. The transfer can occur *in vitro*, *ex vivo*, *in vivo,* or combinations thereof.

In a highly preferred aspect, the delivery system is a retroviral delivery system.

Retroviral vector systems have been proposed as a delivery system for *inter alia* the transfer of a NOI to one or more sites of interest. The transfer can occur *in vitro*, *ex vivo*, *in vivo,* or combinations thereof. Retroviral vector systems have even been exploited to study various aspects of the retrovirus life cycle, including receptor usage, reverse transcription and RNA packaging (reviewed by Miller, 1992 Curr Top Microbiol Immunol 158:1-24).

As used herein the term "vector system" also includes a vector particle capable of transducing a recipient cell with an NOI.

A vector particle includes the following components: a vector genome, which may contain one or more NOIs, a nucleocapsid encapsidating the nucleic acid, and a membrane surrounding the nucleocapsid.

The term "nucleocapsid" refers to at least the group specific viral core proteins (gag) and the viral polymerase (pol) of a retrovirus genome. These proteins encapsidate the packagable sequences and are themselves further surrounded by a membrane containing an envelope glycoprotein.

Once within the cell, the RNA genome from a retroviral vector particle is reverse transcribed into DNA and integrated into the DNA of the recipient cell.

The term "vector genome" refers to both to the RNA construct present in the retroviral vector particle and the integrated DNA construct. The term also embraces a separate or isolated DNA construct capable of encoding such an RNA genome. A retroviral or lentiviral genome should comprise at least one component part derivable from a retrovirus or a lentivirus. The term "derivable" is used in its normal sense as meaning a nucleotide sequence or a part thereof which need not necessarily be obtained from a virus such as a lentivirus but instead could be derived therefrom. By way of example, the sequence may be prepared synthetically or by use of recombinant DNA techniques. Preferably the genome comprises a *psi* region (or an analogous component which is capable of causing encapsidation).

The viral vector genome is preferably "replication defective" by which we mean that the genome does not comprise sufficient genetic information alone to enable independent replication to produce infectious viral particles within the recipient cell. In a preferred embodiment, the genome lacks a functional *env, gag* or *pol* gene. If a highly preferred embodiment the genome lacks *env, gag* and *pol* genes.

The viral vector genome may comprise some or all of the long terminal repeats (LTRs). Preferably the genome comprises at least part of the LTRs or an analogous sequence which is capable of mediating proviral integration, and transcription. The sequence may also comprise or act as an enhancer-promoter sequence.

It is known that the separate expression of the components required to produce a retroviral vector particle on separate DNA sequences cointroduced into the same cell will yield retroviral particles carrying defective retroviral genomes that carry therapeutic genes (e.g. Reviewed by Miller 1992). This cell is referred to as the producer cell (see below).

There are two common procedures for generating producer cells. In one, the sequences encoding retroviral Gag, Pol and Env proteins are introduced into the cell and stably integrated into the cell genome; a stable cell line is produced which is referred to as the packaging cell line. The packaging cell line produces the proteins required for packaging retroviral RNA but it cannot bring about encapsidation due to the lack of a *psi* region. However, when a vector genome (having a *psi* region) is introduced into the packaging cell line, the helper proteins can package the *psi*-positive recombinant vector RNA to produce the recombinant virus stock. This can be used to transduce the NOI into recipient cells. The recombinant virus whose genome lacks all genes required to make viral proteins can infect only once and cannot propagate. Hence, the NOI is introduced into the host cell genome without the generation of potentially harmful retrovirus. A summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 449).

The present invention also provides a packaging cell line comprising a viral vector genome which is capable of producing a vector system useful in the first aspect of the invention. For example, the packaging cell line may be transduced with a viral vector system comprising the genome or transfected with a plasmid carrying a DNA construct capable of encoding the RNA genome. The present invention also provides a kit for producing a retroviral vector system useful in the first aspect of the invention which comprises a packaging cell and a retroviral vector genome.

The second approach is to introduce the three different DNA sequences that are required to produce a retroviral vector particle i.e. the *env* coding sequences, the *gag-pol* coding sequence and the defective retroviral genome containing one or more NOIs into the cell at the same time by transient transfection and the procedure is referred to as transient triple

transfection (Landau & Littman 1992; Pear et al 1993). The triple transfection procedure has been optimised (Soneoka et al 1995; Finer et al 1994). WO 94/29438 describes the production of producer cells *in vitro* using this multiple DNA transient transfection method. WO 97/27310 describes a set of DNA sequences for creating retroviral producer cells either *in vivo* or *in vitro* for re-implantation.

The components of the viral system which are required to complement the vector genome may be present on one or more "producer plasmids" for transfecting into cells.

The present invention also provides a kit for producing a retroviral vector system useful in the first aspect of the invention, comprising

(i) a viral vector genome which is incapable of encoding one or more proteins which are required to produce a vector particle;

(ii) one or more producer plasmid(s) capable of encoding the protein which is not encoded by (i); and optionally

(iii) a cell suitable for conversion into a producer cell.

In a preferred embodiment, the viral vector genome is incapable of encoding the proteins gag, pol and env. Preferably the kit comprises one or more producer plasmids encoding env, gag and pol, for example, one producer plasmid encoding env and one encoding gag-pol. Preferably the gag-pol sequence is codon optimised for use in the particular producer cell (see below).

The present invention also provides a producer cell expressing the vector genome and the producer plasmid(s) capable of producing a retroviral vector system useful in the present invention.

Preferably the retroviral vector system used in the first aspect of the present invention is a self-inactivating (SIN) vector system.

By way of example, self-inactivating retroviral vector systems have been constructed by deleting the transcriptional enhancers or the enhancers and promoter in the U3 region of the 3′ LTR. After a round of vector reverse transcription and integration, these changes are copied into both the 5′ and the 3′ LTRs producing a transcriptionally inactive provirus. However, any promoter(s) internal to the LTRs in such vectors will still be transcriptionally active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes. Such effects include increased transcription or suppression of transcription. This strategy can also be used to eliminate downstream transcription from the 3′ LTR into genomic DNA. This is of particular concern in human gene therapy where it may be important to prevent the adventitious activation of an endogenous oncogene.

Preferably a recombinase assisted mechanism is used which facilitates the production of high titre regulated lentiviral vectors from the producer cells of the present invention.

As used herein, the term "recombinase assisted system" includes but is not limited to a system using the Cre recombinase / loxP recognition sites of bacteriophage P1 or the site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs).

The site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs) has been configured into DNA constructs in order to generate high level producer cell lines using recombinase-assisted recombination events (Karreman et al (1996) NAR 24:1616-1624). A similar system has been developed using the Cre recombinase / loxP recognition sites of bacteriophage P1 (see PCT/GB00/03837; Vanin et al (1997) J. Virol 71:7820-7826). This was configured into a lentiviral genome such that high titre lentiviral producer cell lines were generated.

By using producer/packaging cell lines, it is possible to propagate and isolate quantities of retroviral vector particles (e.g. to prepare suitable titres of the retroviral vector particles) for

subsequent transduction of, for example, a site of interest (such as adult brain tissue). Producer cell lines are usually better for large scale production of vector particles.

Transient transfection has numerous advantages over the packaging cell method. In this regard, transient transfection avoids the longer time required to generate stable vector-producing cell lines and is used if the vector genome or retroviral packaging components are toxic to cells. If the vector genome encodes toxic genes or genes that interfere with the replication of the host cell, such as inhibitors of the cell cycle or genes that induce apoptosis, it may be difficult to generate stable vector-producing cell lines, but transient transfection can be used to produce the vector before the cells die. Also, cell lines have been developed using transient infection that produce vector titre levels that are comparable to the levels obtained from stable vector-producing cell lines (Pear et al 1993, PNAS 90:8392-8396).

Producer cells/packaging cells can be of any suitable cell type. Producer cells are generally mammalian cells but can be, for example, insect cells.

As used herein, the term "producer cell" or "vector producing cell" refers to a cell which contains all the elements necessary for production of retroviral vector particles.

Preferably, the producer cell is obtainable from a stable producer cell line.

Preferably, the producer cell is obtainable from a derived stable producer cell line.

Preferably, the producer cell is obtainable from a derived producer cell line.

As used herein, the term "derived producer cell line" is a transduced producer cell line which has been screened and selected for high expression of a marker gene. Such cell lines support high level expression from the retroviral genome. The term "derived producer cell line" is used interchangeably with the term "derived stable producer cell line" and the term "stable producer cell line.

Preferably the derived producer cell line includes but is not limited to a retroviral and/or a lentiviral producer cell.

Preferably the derived producer cell line is an HIV or EIAV producer cell line, more preferably an EIAV producer cell line.

Preferably the envelope protein sequences, and nucleocapsid sequences are all stably integrated in the producer and/or packaging cell. However, one or more of these sequences could also exist in episomal form and gene expression could occur from the episome.

As used herein, the term "packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant virus which are lacking in the RNA genome. Typically, such packaging cells contain one or more producer plasmids which are capable of expressing viral structural proteins (such as *gag-pol* and *env,* which may be codon optimised) but they do not contain a packaging signal.

The term "packaging signal" which is referred to interchangeably as "packaging sequence" or "*psi*" is used in reference to the non-coding, *cis*-acting sequence required for encapsidation of retroviral RNA strands during viral particle formation. In HIV-1, this sequence has been mapped to loci extending from upstream of the major splice donor site (SD) to at least the *gag* start codon.

Packaging cell lines may be readily prepared (see also WO 92/05266), and utilised to create producer cell lines for the production of retroviral vector particles. As already mentioned, a summary of the available packaging lines is presented in "Retroviruses" (as above).

Also as discussed above, simple packaging cell lines, comprising a provirus in which the packaging signal has been deleted, have been found to lead to the rapid production of

undesirable replication competent viruses through recombination. In order to improve safety, second generation cell lines have been produced wherein the 3'LTR of the provirus is deleted. In such cells, two recombinations would be necessary to produce a wild type virus. A further improvement involves the introduction of the *gag-pol* genes and the *env* gene on separate constructs so-called third generation packaging cell lines. These constructs are introduced sequentially to prevent recombination during transfection.

Preferably, the packaging cell lines are second generation packaging cell lines.

Preferably, the packaging cell lines are third generation packaging cell lines.

In these split-construct, third generation cell lines, a further reduction in recombination may be achieved by changing the codons. This technique, based on the redundancy of the genetic code, aims to reduce homology between the separate constructs, for example between the regions of overlap in the *gag-pol* and *env* open reading frames.

The packaging cell lines are useful for providing the gene products necessary to encapsidate and provide a membrane protein for a high titre vector particle production. The packaging cell may be a cell cultured *in vitro* such as a tissue culture cell line. Suitable cell lines include but are not limited to mammalian cells such as murine fibroblast derived cell lines or human cell lines. Preferably the packaging cell line is a human cell line, such as for example: HEK293, 293-T, TE671, HT1080.

Alternatively, the packaging cell may be a cell derived from the individual to be treated such as a monocyte, macrophage, blood cell or fibroblast. The cell may be isolated from an individual and the packaging and vector components administered *ex vivo* followed by re-administration of the autologous packaging cells.

It is highly desirable to use high-titre virus preparations in both experimental and practical applications. Techniques for increasing viral titre include using a *psi* plus packaging signal as discussed above and concentration of viral stocks.

As used herein, the term "high titre" means an effective amount of a retroviral vector or particle which is capable of transducing a target site such as a cell.

As used herein, the term "effective amount" means an amount of a regulated retroviral or lentiviral vector or vector particle which is sufficient to induce expression of the NOIs at a target site.

A high-titre viral preparation for a producer/packaging cell is usually of the order of 10^5 to 10^7 t.u. per ml. (The titer is expressed in transducing units per ml (t.u./ml) as titred on a standard D17 cell line). For transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^9 t.u./ml, more preferably at least 10^9 t.u./ml.

The expression products encoded by the NOIs may be proteins which are secreted from the cell. Alternatively the NOI expression products are not secreted and are active within the cell. For some applications, it is preferred for the NOI expression product to demonstrate a bystander effect or a distant bystander effect; that is the production of the expression product in one cell leading to the modulation of additional, related cells, either neighbouring or distant (e.g. metastatic), which possess a common phenotype.

The presence of a sequence termed the central polypurine tract (cPPT) may improve the efficiency of gene delivery to non-dividing cells (see WO 00/31200). This *cis*-acting element is located, for example, in the EIAV polymerase coding region element. Preferably the genome of the vector system used in the present invention comprises a cPPT sequence.

In addition, or in the alternative, the viral genome may comprise a post-translational regulatory element and/or a translational enhancer.

The NOIs may be operatively linked to one or more promoter/enhancer elements. Transcription of one or more NOI may be under the control of viral LTRs or alternatively promoter-enhancer elements can be engineered in with the transgene. Preferably the promoter is a strong promoter such as CMV. The promoter may be a regulated promoter. The promoter may be tissue-specific. In a preferred embodiment the promoter is glial cell-specific. In another preferred embodiment the promoter is neuron-specific.

MINIMAL SYSTEMS

It has been demonstrated that a primate lentivirus minimal system can be constructed which requires none of the HIV/SIV additional genes *vif, vpr, vpx, vpu, tat, rev* and *nef* for either vector production or for transduction of dividing and non-dividing cells. It has also been demonstrated that an EIAV minimal vector system can be constructed which does not require *S2* for either vector production or for transduction of dividing and non-dividing cells. The deletion of additional genes is highly advantageous. Firstly, it permits vectors to be produced without the genes associated with disease in lentiviral (e.g. HIV) infections. In particular, *tat* is associated with disease. Secondly, the deletion of additional genes permits the vector to package more heterologous DNA. Thirdly, genes whose function is unknown, such as *S2*, may be omitted, thus reducing the risk of causing undesired effects. Examples of minimal lentiviral vectors are disclosed in WO-A-99/32646 and in WO-A-98/17815.

Thus, preferably, the delivery system used in the invention is devoid of at least *tat* and *S2* (if it is an EIAV vector system), and possibly also *vif, vpr, vpx, vpu* and *nef.* More preferably, the systems of the present invention are also devoid of *rev.* Rev was previously thought to be essential in some retroviral genomes for efficient virus production. For example, in the case of HIV, it was thought that *rev* and RRE sequence should be included. However, it has been found that the requirement for *rev* and RRE

can be reduced or eliminated by codon optimisation (see below) or by replacement with other functional equivalent systems such as the MPMV system. As expression of the codon optimised *gag-pol* is REV independent, RRE can be removed from the *gag-pol* expression cassette, thus removing any potential for recombination with any RRE contained on the vector genome.

In a preferred embodiment the viral genome of the first aspect of the invention lacks the Rev response element (RRE).

In a preferred embodiment, the system used in the present invention is based on a so-called "minimal" system in which some or all of the additional genes have be removed.

CODON OPTIMISATION

Codon optimisation has previously been described in WO99/41397. Different cells differ it their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corrsponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available.

Many viruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms.

Codon optimisation has a number of other advantages. By virtue of alterations in their sequences, the nucleotide sequences encoding the packaging components of the viral particles required for assembly of viral particles in the producer cells/packaging cells

have RNA instability sequences (INS) eliminated from them. At the same time, the amino acid sequence coding sequence for the packaging components is retained so that the viral components encoded by the sequences remain the same, or at least sufficiently similar that the function of the packaging components is not compromised. Codon optimisation also overcomes the Rev/RRE requirement for export, rendering optimised sequences Rev independent. Codon optimisation also reduces homologous recombination between different constructs within the vector system (for example between the regions of overlap in the gag-pol and env open reading frames). The overall effect of codon optimisation is therefore a notable increase in viral titre and improved safety.

In one embodiment only codons relating to INS are codon optimised. However, in a much more preferred and practical embodiment, the sequences are codon optimised in their entirety, with the exception of the sequence encompassing the frameshift site.

The *gag-pol* gene comprises two overlapping reading frames encoding the gag-pol proteins. The expression of both proteins depends on a frameshift during translation. This frameshift occurs as a result of ribosome "slippage" during translation. This slippage is thought to be caused at least in part by ribosome-stalling RNA secondary structures. Such secondary structures exist downstream of the frameshift site in the *gag-pol* gene. For HIV, the region of overlap extends from nucleotide 1222 downstream of the beginning of *gag* (wherein nucleotide 1 is the A of the *gag* ATG) to the end of *gag* (nt 1503). Consequently, a 281 bp fragment spanning the frameshift site and the overlapping region of the two reading frames is preferably not codon optimised. Retaining this fragment will enable more efficient expression of the gag-pol proteins.

For EIAV the beginning of the overlap has been taken to be nt 1262 (where nucleotide 1 is the A of the *gag* ATG). The end of the overlap is at 1461 bp. In order to ensure that the frameshift site and the *gag-pol* overlap are preserved, the wild type sequence has been retained from nt 1156 to 1465.

Derivations from optimal codon usage may be made, for example, in order to accommodate convenient restriction sites, and conservative amino acid changes may be introduced into the gag-pol proteins.

In a highly preferred embodiment, codon optimisation was based on lightly expressed mammalian genes. The third and sometimes the second and third base may be changed.

Due to the degenerate nature of the Genetic Code, it will be appreciated that numerous *gag-pol* sequences can be achieved by a skilled worker. Also there are many retroviral variants described which can be used as a starting point for generating a codon optimised *gag-pol* sequence. Lentiviral genomes can be quite variable. For example there are many quasi-species of HIV-1 which are still functional. This is also the case for EIAV. These variants may be used to enhance particular parts of the transduction process. Examples of HIV-1 variants may be found at http://hiv-web.lanl.gov. Details of EIAV clones may be found at the NCBI database: http://www.ncbi.nlm.nih.gov.

The strategy for codon optimised *gag-pol* sequences can be used in relation to any retrovirus. This would apply to all lentiviruses, including EIAV, FIV, BIV, CAEV, VMR, SIV, HIV-1 and HIV-2. In addition this method could be used to increase expression of genes from HTLV-1, HTLV-2, HFV, HSRV and human endogenous retroviruses (HERV), MLV and other retroviruses.

Codon optimisation can render *gag-pol* expression Rev independent. In order to enable the use of anti-*rev* or RRE factors in the retroviral vector, however, it would be necessary to render the viral vector generation system totally Rev/RRE independent. Thus, the genome also needs to be modified. This is achieved by optimising vector genome components. Advantageously, these modifications also lead to the production of a safer system absent of all additional proteins both in the producer and in the transduced cell.

As described above, the packaging components for a retroviral vector include expression products of *gag, pol* and *env* genes. In addition, efficient packaging depends on a short

sequence of 4 stem loops followed by a partial sequence from *gag* and *env* (the "packaging signal"). Thus, inclusion of a deleted *gag* sequence in the retroviral vector genome (in addition to the full *gag* sequence on the packaging construct) will optimise vector titre. To date efficient packaging has been reported to require from 255 to 360 nucleotides of *gag* in vectors that still retain *env* sequences, or about 40 nucleotides of *gag* in a particular combination of splice donor mutation, *gag* and *env* deletions. It has surprisingly been found that a deletion of all but the N-termnial 360 or so nucleotides in *gag* leads to an increase in vector titre. Thus, preferably, the retroviral vector genome includes a *gag* sequence which comprises one or more deletions, more preferably the gag sequence comprises about 360 nucleotides derivable from the N-terminus.

PSEUDOTYPING

In the design of retroviral vector systems it is desirable to engineer particles with different target cell specificities to the native virus, to enable the delivery of genetic material to an expanded or altered range of cell types. One manner in which to achieve this is by engineering the virus envelope protein to alter its specificity. Another approach is to introduce a heterologous envelope protein into the vector particle to replace or add to the native envelope protein of the virus.

The term pseudotyping means incorporating in at least a part of, or substituting a part of, or replacing all of, an *env* gene of a viral genome with a heterologous *env* gene, for example an *env* gene from another virus. Pseudotyping is not a new phenomenon and examples may be found in WO 99/61639, WO-A-98/05759, WO-A-98/05754, WO-A-97/17457, WO-A-96/09400, WO-A-91/00047 and Mebatsion *et al* 1997 Cell 90, 841-847.

Pseudotyping can improve retroviral vector stability and transduction efficiency. A pseudotype of murine leukemia virus packaged with lymphocytic choriomeningitis virus (LCMV) has been described (Miletic et al (1999) J. Virol. 73:6114-6116) and shown to

be stable during ultracentrifugation and capable of infecting several cell lines from different species.

In the present invention the vector system may be pseudotyped with at least a part of a mutant rabies G envelope protein, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

Thus, the retroviral delivery system used in the first aspect of the invention comprises a first nucleotide sequence coding for at least a part of an envelope protein; and one or more other nucleotide sequences derivable from a retrovirus that ensure transduction by the retroviral delivery system; wherein the first nucleotide sequence is heterologous with respect to at least one of the other nucleotide sequences; and wherein the first nucleotide sequence codes for at least a part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or at least a part of a CVS protein or a mutant, variant, homologue or fragment thereof.

There is thus provided the use of a retroviral delivery system comprising a heterologous *env* region, wherein the heterologous *env* region comprises at least a part of a rabies G protein or a mutant, variant, homologue or fragment thereof or at least a part of a CVS protein or a mutant, variant, homologue or fragment thereof.

The heterologous env region may be encoded by a gene which is present on a producer plasmid. The producer plasmid may be present as part of a kit for the production of retroviral vector particles suitable for use in the first aspect of the invention.

RABIES G PROTEIN

In the present invention the vector system may be pseudotyped with at least a part of a mutant rabies G protein or a variant, homologue or fragment thereof.

Teachings on the rabies G protein, as well as mutants thereof, may be found in in WO 99/61639 and well as Rose *et al.*, 1982 J. Virol. 43: 361-364, Hanham *et al.*, 1993 J. Virol.,67, 530-542, Tuffereau *et al.*,1998 J. Virol., 72, 1085-1091, Kucera *et al.*, 1985 J. Virol 55, 158-162, Dietzschold *et al.*, 1983 PNAS 80, 70-74, Seif *et al.*, 1985 J.Virol., 53, 926-934, Coulon *et al.*,1998 J. Virol., 72, 273-278, Tuffereau *et al.*,1998 J. Virol., 72, 1085-10910, Burger *et al.*, 1991 J.Gen. Virol. 72. 359-367, Gaudin *et al* 1995 J Virol 69, 5528-5534, Benmansour *et al* 1991 J Virol 65, 4198-4203, Luo *et al* 1998 Microbiol Immunol 42, 187-193, Coll 1997 Arch Virol 142, 2089-2097, Luo *et al* 1997 Virus Res 51, 35-41 Luo *et al* 1998 Microbiol Immunol 42, 187-193, Coll 1995 Arch Virol 140, 827-851, Tuchiya *et al* 1992 Virus Res 25, 1-13, Morimoto *et al* 1992 Virology 189, 203-216, Gaudin *et al* 1992 Virology 187, 627-632, Whitt *et al* 1991 Virology 185, 681-688, Dietzschold *et al* 1978 J Gen Virol 40, 131-139, Dietzschold *et al* 1978 Dev Biol Stand 40, 45-55, Dietzschold *et al* 1977 J Virol 23, 286-293, and Otvos *et al* 1994 Biochim Biophys Acta 1224, 68-76. A rabies G protein is also described in EP-A-0445625.

The use of rabies G protein provides vectors which, *in vivo,* preferentially transduce targeted cells which rabies virus preferentially infects. This includes in particular neuronal target cells *in vivo*. For a neuron-targeted vector, rabies G from a pathogenic strain of rabies such as ERA may be particularly effective. On the other hand rabies G protein confers a wider target cell range *in vitro* including nearly all mammalian and avian cell types tested (Seganti *et al.*, 1990 Arch Virol. 34,155-163; Fields *et al.*, 1996 Fields Virology, Third Edition, vol.2, Lippincott-Raven Publishers, Philadelphia, New York).

The tropism of the pseudotyped vector particles may be modified by the use of a mutant rabies G which is modified in the extracellular domain. Rabies G protein has the advantage of being mutatable to restrict target cell range. The uptake of rabies virus by target cells *in vivo* is thought to be mediated by the acetylcholine receptor (AchR) but there may be other receptors to which in binds *in vivo* (Hanham *et al.*, 1993 J. Virol.,67, 530-542; Tuffereau *et al.*,1998 J. Virol., 72, 1085-1091). It is thought that multiple receptors are used in the nervous system for viral entry, including NCAM (Thoulouze et

al (1998) J. Virol 72(9):7181-90) and p75 Neurotrophin receptor (Tuffereau C et al (1998) EMBO J 17(24) 7250-9).

The effects of mutations in antigenic site III of the rabies G protein on virus tropism have been investigated, this region it is reported is not thought to be involved in the binding of the virus to the acetylcholine receptor (Kucera *et al.,* 1985 J. Virol 55, 158-162; Dietzschold *et al.,* 1983 Proc Natl Acad Sci 80, 70-74; Seif *et al.,* 1985 J.Virol., 53, 926-934; Coulon *et al.*,1998 J. Virol., 72, 273-278; Tuffereau *et al.*,1998 J. Virol., 72, 1085-10910). For example it has been reported that a mutation of the arginine at amino acid 333 in the mature protein to glutamine (i.e. ERAsm) can be used to restrict viral entry to olfactory and peripheral neurons *in vivo* while reducing propagation to the central nervous system. It has also been reported that these viruses were able to penetrate motor neurons and sensory neurons as efficiently as the wild type virus, yet transneuronal transfer did not occur (Coulon *et al.*, 1989, J. Virol. 63, 3550-3554). Viruses in which amino acid 330 has been mutated are further attenuated (i.e. ERAdm), were reported as being unable to infect either motor neurons or sensory neurons after intra-muscular injection (Coulon *et al.*,1998 J. Virol., 72, 273-278).

Alternatively or additionally, rabies G proteins from laboratory passaged strains of rabies may be used. These can be screened for alterations in tropism. Such strains include the following:

Genbank accession number	**Rabies Strain**
J02293	ERA
U52947	COSRV
U27214	NY 516
U27215	NY771
U27216	FLA125
U52946	SHBRV
M32751	HEP-Flury

By way of example, the ERA strain is a pathogenic strain of rabies and the rabies G protein from this strain can be used for transduction of neuronal cells. The sequence of rabies G from the ERA strains is in the GenBank database (accession number J02293). This protein has a signal peptide of 19 amino acids and the mature protein begins at the lysine residue 20 amino acids from the translation initiation methionine. The HEP-Flury strain contains the mutation from arginine to glutamine at amino acid position 333 in the mature protein which correlates with reduced pathogenicity and which can be used to restrict the tropism of the viral envelope.

WO 99/61639 discloses the nucleic and amino acid sequences for a rabies virus strain ERA (Genbank locus RAVGPLS, accession M38452).

CVS

In the present invention the vector system may be pseudotyped with at least part of a CVS (Challenge Standard Virus) protein, and in particular the CVS rabies virus glycoprotein G, or a mutant, variant, homologue or fragment thereof.

Teachings on CVS can be found in US Patent No. 5,348,741.

It will also be appreciated that CVS glycoproteins from laboratory passaged strains of CVS may be used. These can be screened for alterations in tropism.

ATCC deposit No. 40280, designated pKB3-JE-13, may conveniently be used in the present invention.

MUTANTS, VARIANTS, HOMOLOGUES AND FRAGMENTS

The vector system is or comprises at least part of a wild-type rabies G protein or a mutant, variant, homologue or fragment thereof and/or a wild-type CVS protein or a mutant, variant, homologue or fragment thereof.

The term "wild type" is used to mean a polypeptide having a primary amino acid sequence which is identical with the native protein (i.e., the viral protein).

The term "mutant" is used to mean a polypeptide having a primary amino acid sequence which differs from the wild type sequence by one or more amino acid additions, substitutions or deletions. A mutant may arise naturally, or may be created artificially (for example by site-directed mutagenesis).Preferably the mutant has at least 90% sequence identity with the wild type sequence. Preferably the mutant has 20 mutations or less over the whole wild-type sequence. More preferably the mutant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

The term "variant" is used to mean a naturally occurring polypeptide which differs from a wild-type sequence. A variant may be found within the same viral strain (i.e. if there is more than one isoform of the protein) or may be found within a different strains. Preferably the variant has at least 90% sequence identity with the wild type sequence. Preferably the variant has 20 mutations or less over the whole wild-type sequence. More preferably the variant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

Here, the term "homologue" means an entity having a certain homology with the wild type amino acid sequence and the wild type nucleotide sequence. Here, the term "homology" can be equated with "identity".

In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98%

identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

In the present context, an homologous sequence is taken to include a nucleotide sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical to the subject sequence. Typically, the homologues will comprise the same sequences that code for the active sites etc. as the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without

penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.*, 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 ibid – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 ibid, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

The present invention also encompasses homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine (hereinafter referred to as Z), diaminobutyric acid ornithine (hereinafter referred to as B), norleucine ornithine (hereinafter referred to as O), pyriylalanine, thienylalanine, naphthylalanine and phenylglycine.

Replacements may also be made by unnatural amino acids include; alpha* and alpha-disubstituted* amino acids, N-alkyl amino acids*, lactic acid*, halide derivatives of natural amino acids such as trifluorotyrosine*, p-Cl-phenylalanine*, p-Br-phenylalanine*, p-I-phenylalanine*, L-allyl-glycine*, ß-alanine*, L-α-amino butyric acid*, L-γ-amino butyric acid*, L-α-amino isobutyric acid*, L-ε-amino caproic acid#, 7-amino heptanoic acid*, L-methionine sulfone#*, L-norleucine*, L-norvaline*, p-nitro-L-phenylalanine*, L-hydroxyproline#, L-thioproline*, methyl derivatives of phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)#, L-Tyr (methyl)*, L-Phe (4-isopropyl)*, L-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid # and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above (relating to homologous or non-homologous substitution), to indicate the hydrophobic nature of the derivative whereas # has been

utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics.

Variant amino acid sequences may include suitable spacer groups that may be inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β-alanine residues. A further form of variation, involves the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino acid residues wherein the α-carbon substituent group is on the residue's nitrogen atom rather than the α-carbon. Processes for preparing peptides in the peptoid form are known in the art, for example Simon RJ et al., PNAS (1992) 89(20), 9367-9371 and Horwell DC, Trends Biotechnol. (1995) 13(4), 132-134.

The term "fragment" indicates that the polypeptide comprises a fraction of the wild-type amino acid sequence. It may comprise one or more large contiguous sections of sequence or a plurality of small sections. The polypeptide may also comprise other elements of sequence, for example, it may be a fusion protein with another protein. Preferably the polypeptide comprises at least 50%, more preferably at least 65%, most preferably at least 80% of the wild-type sequence.

With respect to function, the mutant, variant, homologue or fragment should be capable of transducing at least part of the brain, a motor neuron or cerebrospinal fluid (CSF0 when used to pseudotype an appropriate vector.

The mutant, variant, homologue or fragment should alternatively or in addition, be capable of conferring the capacity for retrograde transport on the vector system.

The vector delivery system used in the present invention may comprise nucleotide sequences that can hybridise to the nucleotide sequence presented herein (including complementary sequences of those presented herein). In a preferred aspect, the present

invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1 SSC) to the nucleotide sequence presented herein (including complementary sequences of those presented herein).

NOIs

In the present invention, preferably the EOI is one or more NOIs (nucleotide sequences of interest) – wherein said NOIs may be delivered to a target cell *in vivo* or *in vitro*.

If the vector system of the present invention is a viral vector system, then it is possible to manipulate the viral genome so that viral genes are replaced or supplemented with one or more NOIs which may be heterologous NOIs.

The term "heterologous" refers to a nucleic acid or protein sequence linked to a nucleic acid or protein sequence to which it is not naturally linked.

In the present invention, the term NOI includes any suitable nucleotide sequence, which need not necessarily be a complete naturally occurring DNA or RNA sequence. Thus, the NOI can be, for example, a synthetic RNA/DNA sequence, a recombinant RNA/DNA sequence (i.e. prepared by use of recombinant DNA techniques), a cDNA sequence or a partial genomic DNA sequence, including combinations thereof. The sequence need not be a coding region. If it is a coding region, it need not be an entire coding region. In addition, the RNA/DNA sequence can be in a sense orientation or in an anti-sense orientation. Preferably, it is in a sense orientation. Preferably, the sequence is, comprises, or is transcribed from cDNA.

The retroviral vector genome may generally comprise LTRs at the 5' and 3' ends, suitable insertion sites for inserting one or more NOI(s), and/or a packaging signal to enable the genome to be packaged into a vector particle in a producer cell. There may even be suitable primer binding sites and integration sites to allow reverse transcription of the vector RNA to DNA, and integration of the proviral DNA into the target cell genome. In

a preferred embodiment, the retroviral vector particle has a reverse transcription system (compatible reverse transcription and primer binding sites) and an integration system (compatible integrase and integration sites).

The NOI may encode a protein of interest ("POI"). In this way, the vector delivery system could be used to examine the effect of expression of a foreign gene on the target cell (such as a TH positive neuron). For example, the retroviral delivery system could be used to screen a cDNA library for a particular effect on the brain, motor neuron or CSF.

For example, one could identify new survival/neuroprotective factors for dopaminergic neurons, which would enable transfected TH+ cells to persist in the presence of an apoptosis-inducing factor.

In accordance with the present invention, suitable NOIs include those that are of therapeutic and/or diagnostic application such as, but not limited to: sequences encoding cytokines, chemokines, hormones, antibodies, anti-oxidant molecules, engineered immunoglobulin-like molecules, a single chain antibody, fusion proteins, enzymes, immune co-stimulatory molecules, immunomodulatory molecules, anti-sense RNA, a transdominant negative mutant of a target protein, a toxin, a conditional toxin, an antigen, a tumour suppresser protein and growth factors, membrane proteins, vasoactive proteins and peptides, anti-viral proteins and ribozymes, and derivatives thereof (such as with an associated reporter group). The NOIs may also encode pro-drug activating enzymes.

The expression products encoded by the NOIs may be proteins which are secreted from the cell. Alternatively the NOI expression products are not secreted and are active within the cell. In either event, it is preferred for the NOI expression product to demonstrate a bystander effect or a distant bystander effect; that is the production of the expression product in one cell leading to the killing of additional, related cells, either neighbouring or distant (e.g. metastatic), which possess a common phenotype.

The NOI or its expression product may act to modulate the biological activity of a compound or a pathway. As used herein the term "modulate" includes for example enhancing or inhibiting biological activity. Such modulation may be direct (e.g. including cleavage of, or competitive binding of another substance to a protein) or indirect (e.g. by blocking the initial production of a protein).

The NOI may be capable of blocking or inhibiting the expression of a gene in the target cell. For example, the NOI may be an antisense sequence. The inhibition of gene expression using antisense technology is well known.

The NOI or a sequence derived therefrom may be capable of "knocking out" the expression of a particular gene in the target cell. There are several "knock out" strategies known in the art. For example, the NOI may be capable of integrating in the genome of a neuron so as to disrupt expression of the particular gene. The NOI may disrupt expression by, for example, introducing a premature stop codon, by rendering the downstream coding sequence out of frame, or by affecting the capacity of the encoded protein to fold (thereby affecting its function).

Alternatively, the NOI may be capable of enhancing or inducing ectopic expression of a gene in the target cell. The NOI or a sequence derived therefrom may be capable of "knocking in" the expression of a particular gene.

In one preferred embodiment, the NOI encodes a ribozyme. Ribozymes are RNA molecules that can function to catalyse specific chemical reactions within cells without the obligatory participation of proteins. For example, group I ribozymes take the form of introns which can mediate their own excision from self-splicing precursor RNA. Other ribozymes are derived from self-cleaving RNA structures which are essential for the replication of viral RNA molecules. Like protein enzymes, ribozymes can fold into secondary and tertiary structures that provide specific binding sites for substrates as well as cofactors, such as metal ions. Examples of such structures include hammerhead,

hairpin or stem-loop, pseudoknot and hepatitis delta antigenomic ribozymes have been described.

Each individual ribozyme has a motif which recognises and binds to a recognition site in a target RNA. This motif takes the form of one or more "binding arms" but generally two binding arms. The binding arms in hammerhead ribozymes are the flanking sequences Helix I and Helix III which flank Helix II. These can be of variable length, usually between 6 to 10 nucleotides each, but can be shorter or longer. The length of the flanking sequences can affect the rate of cleavage. For example, it has been found that reducing the total number of nucleotides in the flanking sequences from 20 to 12 can increase the turnover rate of the ribozyme cleaving a HIV sequence, by 10-fold (Goodchild, JVK, 1991 Arch Biochem Biophys 284: 386-391). A catalytic motif in the ribozyme Helix II in hammerhead ribozymes cleaves the target RNA at a site which is referred to as the cleavage site. Whether or not a ribozyme will cleave any given RNA is determined by the presence or absence of a recognition site for the ribozyme containing an appropriate cleavage site.

Each type of ribozyme recognizes its own cleavage site. The hammerhead ribozyme cleavage site has the nucleotide base triplet GUX directly upstream where G is guanine, U is uracil and X is any nucleotide base. Hairpin ribozymes have a cleavage site of BCUGNYR, where B is any nucleotide base other than adenine, N is any nucleotide, Y is cytosine or thymine and R is guanine or adenine. Cleavage by hairpin ribozymes takes places between the G and the N in the cleavage site.

More details on ribozymes may be found in "Molecular Biology and Biotechnology" (Ed. RA Meyers 1995 VCH Publishers Inc p831-8320 and in "Retroviruses" (Ed. JM Coffin et al 1997 Cold Spring Harbour Laboratory Press pp 683).

Expression of the ribozyme may be induced in all cells, but will only exert an effect in those in which the target gene transcript is present.

Alternatively, instead of preventing the association of the components directly, the substance may suppress the biologically available amount of a polypeptide of the invention. This may be by inhibiting expression of the component, for example at the level of transcription, transcript stability, translation or post-translational stability. An example of such a substance would be antisense RNA or double-stranded interfering RNA sequences which suppresses the amount of mRNA biosynthesis.

In another preferred embodiment, the NOI comprises an siRNA. Post-transcriptional gene silencing (PTGS) mediated by double-stranded RNA (dsRNA) is a conserved cellular defence mechanism for controlling the expression of foreign genes. It is thought that the random integration of elements such as transposons or viruses causes the expression of dsRNA which activates sequence-specific degradation of homologous single-stranded mRNA or viral genomic RNA. The silencing effect is known as RNA interference (RNAi). The mechanism of RNAi involves the processing of long dsRNAs into duplexes of 21-25 nucleotide (nt) RNAs. These products are called small interfering or silencing RNAs (siRNAs) which are the sequence-specific mediators of mRNA degradation. In differentiated mammalian cells dsRNA >30bp has been found to activate the interferon response leading to shut-down of protein synthesis and non-specific mRNA degradation. However this response can be bypassed by using 21nt siRNA duplexes allowing gene function to be analysed in cultured mammalian cells.

In one embodiment an RNA polymerase III promoter, e.g., U6, whose activity is regulated by the presence of tetracycline may be used to regulate expression of the siRNA.

In another embodiment the NOI comprises a micro-RNA. Micro-RNAs are a very large group of small RNAs produced naturally in organisms, at least some of which regulate the expression of target genes. Founding members of the micro-RNA family are *let-7* and *lin-4*. The *let-7* gene encodes a small, highly conserved RNA species that regulates the expression of endogenous protein-coding genes during worm development. The active RNA species is transcribed initially as an ~70nt precursor, which is post-

transcriptionally processed into a mature ~21nt form. Both *let-7* and *lin-4* are transcribed as hairpin RNA precursors which are processed to their mature forms by Dicer enzyme.

In a further embodiment the NOI comprises double-stranded interfering RNA in the form of a hairpin. The short hairpin may be expressed from a single promoter, e.g., U6. In an alternative embodiment an effective RNAi may be mediated by incorporating two promoters, e.g., U6 promoters, one expressing a region of sense and the other the reverse complement of the same sequence of the target. In a further embodiment effective or double-stranded interfering RNA may be mediated by using two opposing promoters to transcribe the sense and antisense regions of the target from the forward and complementary strands of the expression cassette.

In another embodiment the NOI may encode a short RNA which may act to redirect splicing ('exon-skipping') or polyadenylation or to inhibit translation.

The NOI may also be an antibody. As used herein, "antibody" includes a whole immunoglobulin molecule or a part thereof or a bioisostere or a mimetic thereof or a derivative thereof or a combination thereof. Examples of a part thereof include: Fab, $F(ab)'_2$, and Fv. Examples of a bioisostere include single chain Fv (ScFv) fragments, chimeric antibodies, bifunctional antibodies.

Transduced target cells which express a particular gene, or which lack the expression of a particular gene have applications in drug discovery and target validation. The expression system could be used to determine which genes have a desirable effect on target cells, such as those genes or proteins which are able to prevent or reverse the triggering of apoptosis in the cells. Equally, if the inhibition or blocking of expression of a particular gene is found to have an undesirable effect on the target cells, this may open up possible therapeutic strategies which ensure that expression of the gene is not lost.

The present invention may therefore be used in conjunction with disease models, such as experimental allergic encephalomyelitis, which is the animal model of Multiple Sclerosis,

and experimental autoimmune neuritis which is the animal model of acute and chronic inflammatory demyelinating polyneuropathy. Other disease models are known to those skilled in the art.

An NOI delivered by the vector delivery system may be capable of immortalising the target cell. A number of immortalisation techniques are known in the art (see for example Katakura Y et al (1998) Methods Cell Biol. 57:69-91).

The vector delivery system can be a non-viral delivery system or a viral delivery system.

In some preferred aspects, the vector delivery system is a viral delivery vector system.

In some further preferred aspects, the vector delivery system is a retroviral vector delivery system.

The term "immortalised" is used herein to cells capable of growing in culture for greater than 10 passages, which may be maintained in continuous culture for greater than about 2 months.

Immortalised motor and sensory neurons and brain cells are useful in experimental procedures, screening programmes and in therapeutic applications. For example, immortalised dopaminergic neurones may be used for transplantation, for example to treat Parkinson's disease.

An NOI delivered by the vector delivery system may be a selection gene, or a marker gene. Many different selectable markers have been used successfully in retroviral vectors. These are reviewed in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 444) and include, but are not limited to, the bacterial neomycin and hygromycin phosphotransferase genes which confer

resistance to G418 and hygromycin respectively; a mutant mouse dihydrofolate reductase gene which confers resistance to methotrexate; the bacterial *gpt* gene which allows cells to grow in medium containing mycophenolic acid, xanthine and aminopterin; the bacterial *hisD* gene which allows cells to grow in medium without histidine but containing histidinol; the multidrug resistance gene (*mdr*) which confers resistance to a variety of drugs; and the bacterial genes which confer resistance to puromycin or phleomycin. All of these markers are dominant selectable and allow chemical selection of most cells expressing these genes.

An NOI delivered by the vector delivery system may be a therapeutic gene - in the sense that the gene itself may be capable of eliciting a therapeutic effect or it may code for a product that is capable of eliciting a therapeutic effect.

The term "mimetic" relates to any chemical which may be a peptide, polypeptide, antibody or other organic chemical which has the same binding specificity as the antibody.

The term "derivative" as used herein includes chemical modification of an antibody. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group.

DISEASES

In general terms the invention is useful for obtaining good distribution of an expressed protein, for example by administering the vector at one site; the protein may be released such that is affects other parts of the brain and CNS.

The vector system used in the present invention is particularly useful in treating and/or preventing a disease which is associated with the death or impaired function of cells of the nervous tissue, such as neurons, CSF and/or brain cells including glial cells. Thus, the vector system is useful in treating and/or preventing neurodegenerative diseases.

In particular, the vector system used in the present invention may be used to treat and/or prevent a disease which is associated with the death or impaired function of motor or sensory neurons.

Diseases which may be treated include, but are not limited to: pain; movement disorders such as Parkinson's disease, motor neuron diseases including amyotrophic lateral schlerosis (ALS or Lou Gehrig's Disease) and Huntington's disease; Alzheimer's Disease; Spinal Muscle Atrophy and Lysosomal Storage Diseases.

Amyotrophic lateral schlerosis (ALS) is a degenerative disorder of motorneurons with a yearly incidence of 1-2 per 100,000. It is characterised by degeneration of motorneurons in the spinal chord, brain stem and motor cortex which leads to wasting and weakness of limb, bulbar and respiratory muscles. Approximately 5-10% of ALS is familial. Genes whose mutations or haplotypes are thought to play a role in disease predisposition include SOD1, ALS2 and VEGF (Lambrechts et al. Nature Genetics 2003; published on line 6 July 2003 (10.1038/ng1211); Oosthuyse et al. Nature Genetics 2001; June; Vol 28 pages 131-138).

In particular, the vector system used in the present invention is useful in treating and/or preventing ALS. In this embodiment, the NOI may be capable of knockdown of SOD1. Other NOI(s) may encode molecules which prevent apoptosis and therefore prevent cells from dying. Suitable molecules include XIAP and NAIP. Alternatively, NOI(s) may encode neurotrophic molecules which stimulate regeneration such as IGF-1, GDNF, VEGF and cardiotrophin (CT1).

Lysosomal Storage Diseases or Glycolipid Storage Disorders are genetic diseases that result when the rate of glycolipid synthesis is not longer balanced with the rate of degradation within the cells. As a result, undegraded glycolipids build up in the lysosomes. Such disorders include Fabry Disease, Niemann-Pick diseases,

Gangliosidosis, Metachromatic Leukodystrophy and many types of Mucopolysaccharidosis.

Spinal Muscular Atrophy (SMA) is a disease of the anterior horn cells and is an autosomal recessive disease. Anterior horn cells are located in the spinal cord. SMA affects the voluntary muscles for activities such as crawling, walking, head and neck control and swallowing. Categories of SMA include: Type I SMA also called *Werdnig-Hoffmann Disease,* Type II, Type III, often referred to as Kugelberg-Welander or Juvenile Spinal Muscular Atrophy, Type IV (Adult Onset) and Adult Onset X-Linked SMA. This form also known as Kennedy's Syndrome or Bulbo-Spinal Muscular Atrophy. SMA is a common motor neuron disease in humans and its most severe form causes death by the age of 2 years. It is caused by mutations in the telomeric survival motor neuron gene, SMN1.

In particular, the vector system used in the present invention is useful in treating and/or preventing SMA. In this embodiment, the NOI may be capable of encoding a gene for replacement of defective SMN1 gene. Other NOI(s) may encode molecules which prevent apoptosis and therefore prevent cells from dying. Suitable molecules include XIAP and NAIP. Alternatively, NOI(s) may encode neurotrophic molecules which stimulate regeneration such as IGF-1, GDNF, VEGF and cardiotrophin (CT1).

In another embodiment, the vector system used in the present invention is useful in treating and/or preventing Parkinson's disease. In this embodiment, the NOI is capable of encoding a neuroprotective molecule. In particular, the NOI(s) may encode molecules which prevent TH-positive neurons from dying or which stimulate regeneration and functional recovery in the damaged nigrostriatal system. In another preferred embodiment, the NOI is capable of encoding an enzyme or enzymes responsible for L-DOPA or dopamine synthesis such as tyrosine hydroxylase.

The vector system of the present invention may also be used in the treatment and/or prevention of an inflammatory neurological disorder including an autoimmune neurological disease.

The inflammatory response evolved to protect organisms against injury and infection. Following an injury or infection a complex cascade of events leads to the delivery of blood-borne leukocytes to sites of injury to kill potential pathogens and promote tissue repair. However, the powerful inflammatory response has the capacity to cause damage to normal tissue, and dysregulation of the innate immune response is involved in different pathologies. It is known that Multiple Sclerosis (MS) is an inflammatory disease of the brain but it has now been suggested that inflammation may significantly contribute to diseases such as stroke, traumatic brain injury, HIV-related dementia, Alzheimer's disease and prion disease.

As mentioned above, MS is a chronic inflammatory disease of the CNS and is presumed to have an autoimmune etiology. MS is believed to be caused by blood-derived T cells specific for CNS antigens. These T cells induce the production in the CNS of antigen-nonspecific mononuclear cells able to destroy oligodendrocytes directly and/or by releasing substances toxic to myelin.

Other autoimmune neurological diseases include the Guillain-Barre syndrome, myasthenia gravis, acute disseminated encephalomyelitis, the stiff-man syndrome, autoimmune neuritis, motor dysfunction, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy, paraproteinaemic neuropathy, autoimmune diseases of the neuromuscular junction and other disorders of the motor unit, inflammatory myopathy, autoimmune myositis, a parameoplastic neurological disorder, neurological complications of connective tissue diseases and vasculitis.

In one embodiment related to the treatment and/prevention of inflammatory disorders, the nucleotide of interest delivered by the vector system used in the present invention encodes an anti-inflammatory molecule, such as an anti-inflammatory cytokine, or a

molecule capable of upregulating the anti-inflammatory molecule. Thus, one embodiment of the present invention relates to a therapeutic approach in neurological inflammatory disorders, such as MS, which involves the delivery of an anti-inflammatory molecule directly to the CNS.

Cytokines which may be useful in the treatment of MS and possible other disorders include IL-1β, IL-2, IL-4, IL-6, IL-1n, IFN-β, IFN-γ, TNF-α, p55TNFR-Ig, p75dTNFR, TGF-β, PDGF-α and NGF. More generally, it will be appreciated that anti-inflammatory cytokines may useful be delivered in accordance with the present invention in the treatment and/or prevention of neurological inflammatory diseases.

Another approach involves the delivery of a nucleotide of interest which inhibits, or encodes a molecule which inhibits, a pro-inflammatory molecule, such as an inflammatory cytokine. Thus the use of inhibitors, such as those described above, e.g. ribozymes, siRNA, antibodies and antisense sequences, is envisaged.

A further approach involves the delivery of myelin proteins and or growth factors for rebuilding and or regenerating the damaged neuron myelin sheath.

The following table summarises a number of examples of diseases that may be treated using the methods and vectors of the present invention along with suggested mechanisms for treatment plus examples of the types of genes that could be modulated in order to treat the disease.

Disease	**Mechanism of treatment**	**Gene(s)**	**Preferred site of therapy**
Pain (cancer)	Interupt signalling	Enkephalin, beta endorphin GDNF,ion channel hyperpolarization	Intraspinal, Intrathecal, amygdala
Pain (diabetic)	As above		DRG, skin

Pain (herpetic neuralgia)		As above	Lesions
Alzheimer's		NGF	cortex
Parkinson's	Dopamine replacement	ADCC, TH, CH1, VMAT2 etc	striatum
Parkinson's	Decrease rate of death of dopaminergic neurones	GDNF, nurturin, other	Striatum, Nigra
Childhood craniopharyngeoma	Avoid diabetic sequellae	Vasopressin	Hypothalamus, Pituitary?
Glioma	Destroy residual tumor after excision	Prodrug activating enzyme (TK, Cyt P450), Angiostatics	Glioma bed
Diabetic Retinopathy	Arrest blood vessel proliferation	Angiostatics, PEDF Flt-1	retina
Macular degeneration	Arrest degeneration	Growth factors	Retina
Retinitis pigmentosa	Arrest degeneration	XIAP, growth factors	Retina, vitreus
Huntington's Disease	Avoid PolyG intracellular effects	CNTF, scAb against polyGlut, CREB factor	striatum
Spinal muscular atrophy	Replace missing protein	SMN1, SMN 2 growth factor: GDNF, IGF-I, VEGF, NT-3, CT-I	Intraspinal Muscle(retrograde)
ALS	Arrest degeneration	SOD1 knockdown (genetic form) by RNAi/antisense, growth factor: GDNF, IGF-I, VEGF, NT-3, CT-I	
Spinal cord regeneration	Promote regrowth, remove inhibitors of regrowth	NT3, antiNogo Antibodies, Growth factors: GDNF, IGF-I	Spinal cord, Intrathecal

Multiple sclerosis	Prevent demyelination	Cytokines	Intrathecal
Lysosomal storage with neurological involvement	Replacement with protein capable of cellular uptake	Beta glucuronidase, Other	Intracerebral, Intraventricular
Stroke	Protect neural tissue in anticipation of second episode	EPO/other using HRE's	Intrathecal

PHARMACEUTICAL COMPOSITIONS

The present invention also provides the use of a vector delivery system in the manufacture of a pharmaceutical composition. The pharmaceutical composition may be used to deliver an EOI, such as an NOI, to a target cell in need of same.

The vector delivery system can be a non-viral delivery system or a viral delivery system.

In some preferred aspects, the vector delivery system is a viral delivery vector system.

In some further preferred aspects, the vector delivery system is a retroviral vector delivery system.

The pharmaceutical composition may be used for treating an individual by gene therapy, wherein the composition comprises or is capable of producing a therapeutically effective amount of a vector system according to the present invention.

The method and pharmaceutical composition of the invention may be used to treat a human or animal subject. Preferably the subject is a mammalian subject. More preferably the subject is a human. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

The composition may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent, any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase the viral entry into the target site (such as for example a lipid delivery system).

Where appropriate, the pharmaceutical compositions can be administered by any one or more of: inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

The vector system used in the present invention may conveniently be administered by direct injection into the patient. For the treatment of neurodegenerative disorders, such as Parkinson's disease, the system may be injected into the brain. The system may be injected directly into any target area of the brain (for example, the striatum or substantia nigra). Alternatively, the system can be injected into a given area, and the target area transduced by retrograde transport of the vector system. Intramuscular injection is particularly preferred as the least invasive method of treatment.

The table above outlines preferred sites for administering therapy by injection and includes intraspinal, intrathecal, amygdala, DRG, skin, sites of lesions of herpetic

neuralgia, cortex, striatum, nigra, hypothalamus, pituitary, glioma bed, retina, vitreus, muscle, spinal cord and intraventricular injection.

TRANSPORT

The present invention provides the use of a vector system comprising at least part of a CVS envelope protein or a mutant, fragment, variant of homologue thereof to transduce a target site, wherein the vector system travels to the site by retrograde transport.

A virus particle may travel in the same direction as a nerve impulse, i.e. from the cell body, along the axon to the axon terminals. This is known as anterograde transport.

The present inventors have shown that vector systems comprising protein of the present invention are capable of retrograde transport, i.e. travelling in the opposite direction. Retrograde transport (or transfer) of a vector means that it is taken up by the axon terminals and travels towards the cell body.

This type of transport is called "Fast transport" and is responsible for the movement of membranous organelles at 50-200 mm per day toward the synapse (anterograde) or back to the cell body (retrograde) (Hirokawa (1997) Curr Opin Neurobiol 7(5):605-614). The precise mechanism of retrograde transport is unknown, however. It is thought to involve transport of the whole viral particle, possibly in association with an internalised receptor. The fact that the present vector systems can be specifically be transported in this manner (as demonstrated herein) suggests that the env protein may be involved.

HSV, adenovirus and hybrid HSV/adeno-associated virus vectors have all been shown to be transported in a retrograde manner in the brain (Horellou and Mallet (1997) Mol Neurobiol 15(2) 241-256; Ridoux *et al* (1994) Brain Res 648:171-175; Constantini *et al* (1999) Human Gene Therapy 10:2481-2494). Injection of Adenoviral vector system expressing glial cell line derived neurotrophic factor (GDNF) into rat striatum allows expression in both dopaminergic axon terminals and cell bodies via retrograde transport

(Horellou and Mallet (1997) as above; Bilang-Bleuel et al (1997) Proc. Natl. Acd. Sci. USA 94:8818-8823).

Retrograde transport can be detected by a number of mechanisms known in the art. In the present examples, a vector system expressing a heterologous gene is injected into the striatum, and expression of the gene is detected in the substantia nigra. It is clear that retrograde transport along the neurons which extend from the substantia nigra to the basal ganglia is responsible for this phenomenon. It is also known to monitor labelled proteins or viruses and directly monitor their retrograde movement using real time confocal microscopy (Hirokawa (1997) as above).

By retrograde transport, it is possible to get expression in both the axon terminals and the cell bodies of transduced neurons. These two parts of the cell may be located in distinct areas of the nervous system. Thus, a single administration (for example, injection) of the vector system of the present invention may transduce many distal sites.

The present invention also provides the use of a vector system of the present invention to transduce a target site which comprises the step of administration of the vector system to an administration site which is distant from the target site to achieve good penetration and biodistribution throughout the CNS. For example, administration to the one area of the brain may give rise to distribution of the EOI is different parts of the brain and/different cell types.

The target site may be any site of interest. It may or may not be anatomically connected to the administration site. The target site may be capable of receiving vector from the administration site by axonal transport, for example anterograde or (more preferably) retrograde transport.

For a given administration site, a number of potential target sites may exist which can be identified using tracers by methods known in the art (Ridoux et al (1994) as above).

For example, intrastriatal injection of CVS/EIAV vectors causes transgene expression in the globus pallidus, cortex, various thalamic nuclei, amygdala, hypothalamus, supraoptic nucleus, deep mesencepthalic nucei, substantia nigra, caudal regions of the brainstem such as the nuclei of the brachium inferior colliculus, paraleminiscal nuclei, genic nucei, parabrachial nuclei, ventral cochlear nuclei and facial nuclei.

A target site is considered to be "distant from the administration" if it is (or is mainly) located in a different region from the administration site. The two sites may be distinguished by their spatial location, morphology and/or function.

In the brain, the basal ganglia consist of several pairs of nuclei, the two members of each pair being located in opposite cerebral hemispheres. The largest nucleus is the corpus striatum which consists of the caudate nucleus and the lentiform nucleus. Each lentiform nucleus is, in turn, subdivided into a lateral part called the putamen and a medial part called the globus pallidus. The substantia nigra and red nuclei of the midbrain and the subthalamic nuclei of the diencephalon are functionally linked to the basal ganglia. Axons from the substantia nigra terminate in the caudate nucleus or the putamen. The subthalamic nuclei connect with the globus pallidus. For conductivity in basal ganglia of the rat see Oorschot (1996) J. Comp. Neurol. 366:580-599.

In a preferred embodiment, the administration site is the striatum of the brain, in particular the caudate putamen. Injection into the putamen can label target sites located in various distant regions of the brain, for example, the globus pallidus, amygdala, subthalamic nucleus or the substantia nigra. Transduction of cells in the pallidus commonly causes retrograde labelling of cells in the thalamus. In a preferred embodiment the (or one of the) target site(s) is the substantia nigra.

Within a given target site, the vector system may transduce a target cell. The target cell may be a cell found in nervous tissue, such as a sensory or motor neuron, astrocyte, oligodendrocyte, microglia or ependymal cell.

The vector system is preferably administered by direct injection. Methods for injection into the brain (in particular the striatum) are well known in the art (Bilang-Bleuel et al (1997) Proc. Acad. Natl. Sci. USA 94:8818-8823; Choi-Lundberg et al (1998) Exp. Neurol.154:261-275; Choi-Lundberg et al (1997) Science 275:838-841; and Mandel et al (1997)) Proc. Acad. Natl. Sci. USA 94:14083-14088). Stereotaxic injections may be given.

As mentioned above, for transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^{10} t.u./ml, more preferably at least 10^9 t.u./ml. (The titer is expressed in transducing units per ml (t.u./ml) as titred on a standard D17 cell line). It has been found that improved dispersion of transgene expression can be obtained by increasing the number of injection sites and decreasing the rate of injection (Horellou and Mallet (1997) as above). Usually between 1 and 10 injection sites are used, more commonly between 2 and 6. For a dose comprising 1-5 x 109 t.u./ml, the rate of injection is commonly between 0.1 and 10 µl/min, usually about 1µl/min.

We have also shown that following administration to the CSF, e.g. using intrathecal delivery, expression of an NOI may be found in various areas of the brain, such as the ependymal and leptomeningeal cells, hippocampus, corpus callosum and septum, and the spinal cord.

TRANSPLANTATION

The present invention also provides an immortalised cell of the CNF such as a sensory or motor neuron or brain cell and its use in transplantation methods.

Grafting protocols using embryonic dopamiergic neurons, equivalent cells from other species, and neural progenitor cells are known (reviewed in Dunnett and Bjorklund

(1999) Nature Vol 399 Supplement pages A32-39). Similar techniques could be used for grafting the cells of the present invention.

FIGURES

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention. The Examples refer to the Figures. In the Figures:

Figures 1 and 2 (SEQ ID NOS:1 and 2) show the polynucleotide and amino acid sequences of ERA wild-type;

Figure 3 (SEQ ID NO:3) shows the polynucleotide sequence of ERAdm;

Figure 4 (SEQ ID NO:4) shows the polynucleotiode sequence of CVS rabies virus glycoprotein;

Figure 5 shows the results of Example 1 and illustrates the transduction efficiency of EIAV-LacZ in the brain following injection into the CSF;

Figure 6 shows the of Example 1 and illustrates the expression of the marker gene LacZ in the spinal cord after injection of EIAV-LacZ into the CSF;

Figure 7 shows the results of Example 2;

Figure 8 shows the results of Example 2;

Figure 9 shows the results of Example 3; and

Figure 10 shows the results of Example 4 using CVS.

EXAMPLES

Details of the EIAV vector system used in the Examples, its production and viral transduction methods can be found in Mazarakis et al (2001) *ibid* and WO02/36170 which are herein incorporated by reference, and in particular the Materials and Methods section of Mazarakis et al (2001) and the Examples section of WO02/36170.

Mutant Rabies G

EIAV vectors were pseudotyped with wild-type and 2 variants of the ERA strain of rabies-G envelopes. The sequence of rabies virus strain ERA is shown in Figures 1 and 2 (SEQ ID NO: 1 and 2). A single mutant of the wild-type ERA strain (**ERAwt**) was generated by replacing arginine at amino acid 333 with glutamine. This mutant, which is naturally occurring and apathogenic in adult mice, was termed **ERAsm**. An additional substitution at amino acid 330 from K to N resulted in a double mutant of ERAwt named **ERAdm**. Both these envelopes were used to pseudotype the EIAV vectors expressing a marker gene LacZ.

In more detail, a partial PCR fragment of the ERAwt that incorporated the 2 amino acid changes was amplified using the following primers:

(5' to 3') CTA CAA CTC AGT CAT GAC TTG GAA TGA GAT CCT CCC CTC AAA AGG GTG TTT AAG AGT TGG GGG GAG G

(5' to 3') CCT TTT GAG GGG AGG ATC TCA TTC CAA GTC ATG ACT GAG TTG TAG TGA GCA TCG GCT TCC ATC AAG GTC

The full-length fragment of the ERAdm (incorporating the 2 amino acid changes) was then amplified using the following primers:

(5' to 3') ACC GTC CTT GAC ACG AAG CT
(5' to 3') GGG GGA GGT GTG GGA GGT TT

The resulting fragment was cloned into pSA91 using appropriate restriction enzymes. Successful clones were sequenced and used to produce EIAV vectors using the transient transfection method.

The sequence of the ERAdm is shown in Figure 3 (SEQ ID NO:3).

CVS

cDNA for CVS (Challenge Virus Standard) rabies virus glycoprotein was obtained from ATCC (ATCC number 40280 designation pKB3-JE-13). The fragment containing the complete coding sequence of the glycoprotein was excised using EcoRI, cloned into pSA91 and sequenced (Bk 1092 pg 75). The sequence is shown in Figure 4 (SEQ ID NO:4).

Viral Transductions

The titres of the various pseudotyped EIAV vectors, as determined by transduction efficiencies in D17 cells, were as follows:

pONY8Z ERAwt	7×10^8 TU/ml
pONY8Z ERAsm	9×10^8 TU/ml
pONY8Z ERAdm	1×10^8 TU/ml
pONY8Z CVS	7×10^8 TU/ml

Example 1 - Injection of EIAV pseudotyped with rabies-G or VSV-G envelopes into the cerebrospinal fluid (CSF) and treatment of MS using an intrathecal route for gene therapy

Stereotaxic administrations were performed under Hypnorm & Hypnovel anesthesia using a 5 µl Hamilton syringe with a 33-gauge blunt tip needle. A total of 8 rats received 10 µl injections of viral vectors into the CSF at coordinates: AP = -0.92; L = 1.4; V = 3.3. The first group of animals (n=4) were injected with EIAV pseudotyped with VSV-G envelope. In the second group (n=4) all the viral vectors were rabies-G pseudotyped. The viral titre was 7×10^8 TU/ml. The lentiviral solution was slowly infused at the speed of 0.2 µl/minute using an infusion pump (World Precision Instruments Inc.). After viral

vector injections, the skin was closed using a 5-0 Vicryl running suture and following surgery, animals were kept warm until recovery was complete. All surgical procedures were approved by the local veterinarian and ethical committee and were carried out according to Home Office regulations.

Following injections into the CSF, the expression of the marker gene LacZ can be demonstrated in different areas of the brain and spinal cord (Figure 5). The rabies-G pseudotyped vectors are able to infect the ependymal and leptomeningeal cells (Figure 5 A-C). Strong bilateral transduction was also observed in the hippocampus (mainly in CA3), corpus collasum and septum (Figure 5 D-I). The virus can also spread to the spinal cord (Figure 6 A-F).

In contrast, no signs of transport or biodistribution are seen with VSV-G pseudotyping.

As demonstrated by these results, the present invention may represent an alternative treatment for inflammatory neurological disorders. Lentivector-mediated delivery of cytokines-encoding genes to the CSF in accordance with the present invention shows the following major advantages : i) the availability of high cytokine levels widely in the CNS; ii) long-term and persistent expression of exogenous genes after incorporation into the DNA of the host cell; and iii) absence of the immune response to the viral particle.

Example 2 - Gene transfer to muscle in neonatal mice using EIAV-rabies-G and EIAV-CVS

To determine if EIAV vectors pseudotyped with rabies-G or CVS envelope is retrogradely transported to the mouse spinal cord, P6 neonatal mice received intramuscular injection of pONY8Z rabies-G viral stock solution (titre 5.7 x 10^8 TU/ml). Seven mice were injected with pONY8Z rabies-G (10 µl, n=2; 20 µl, n=2; 30 µl, n=3). The second group of mice were injected with pONY8Z CVS (titre 7 x 10^8 TU/ml, n=3, volume injected = 30 µl).

The results are shown in Figures 7 and 8 and experiment demonstrates that a large number of motor neurons (MN) were retrogradely transduced after injection of the viral particles in the gastrocnemius muscle. In the present study 10-12 MN (~50% of MN) per section were X-gal-positive in pONY8Z-rabies-G injected mice (Fig 7). In EIAV-CVS injected animals, 7-8 MN per section were x-gal positive (Fig 8). Transduced cells were found to be localised in the ventral horn and only on one side. Examination of the morphology of transduced cells suggested that these cells were motoneurones (cells with large size). β-gal immunostaining was also performed. Muscle cells were also transduced In EIAV-rabies-G injected animals (Fig 7).

Example 3 - Gene transfer to rat spinal cord using EIAV-CVS

For intraspinal injection anesthetized 2 month old rats are placed in a stereotaxic frame and their spinal cords are immobilized using a spinal adaptor (Stoelting Co., IL, USA) and injected into the lumbar spinal cord following laminectomy with 1 µl of pONY8.0Z vector pseudotyped with CVS (n= 3) (7×10^8 T.U./ml) at one site. Injections, controlled by an infusion pump (World Precision Instruments Inc., Sarasota, USA), are at 0.1 µl per minute through a 10 µl Hamilton syringe fitted with a 33 gauge needle. Following injection, the needle is left in place for 5 minutes before being retrieved. Four weeks after viral injection animals were perfused transcardially with 4% w/v paraformaldehyde. The spinal cord and brain were dissected out and analysed X-gal reaction.

The results from this experiment are described in Figure 9. Injection of EIAV-Lac CVS into the spinal cord induce storng transdcution in the injected side with retrograde transport to the contralateral side of the spinal cord. Interestingly motor neurons in the brain stem and cortex were transduced by retrograde transport (Figure 9).

Example 4 - Injection of EIAV vectors pseudotyped with CVS envelope into the striatum

Approximately 2×10^6 TU of each vector was slowly infused into the striatum of adult male Wistar rats (300g) using the stereotaxic coordinates AP 0 mm, ML 3.5 mm, DV

4.75mm and left for 2 or 4 weeks. The rats were then sacrificed and transcardially perfused with 4% paraformaldehyde. Following an overnight incubation in 4% paraformaldehyde, the brains were cryoprotected in 30% sucrose for at least 3 days, after which they were frozen and cut into 40μm coronal sections. X-gal staining and immunohistochemistry were performed.

As shown in Figure 10, when EIAV vectors pseudotyped with the CVS envelope was injected into the striatum, there was strong expression in the globus pallidus. Retrograde transport was observed in the cortex, various thalamic nuclei, amygdala, hypothalamus, supraoptic nucleus, deep mesencephalic nuclei and substantia nigra. In addition retrograde transport to the caudal regions of the brainstem was observed. In this region, various nuclei such as the nuclei of the brachium inferior colliculus, paraleminiscal nuclei, genic nuclei, parabrachial nuclei, ventral cochlear nuclei and facial nuclei were positive for X-gal staining.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biology or related fields are intended to be within the scope of the following claims.

Claims

1. Use of a vector system to transduce a target site, wherein the vector system travels to the target site by diffusion, and wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof, and further wherein the target site is at least part of the central nervous system.

2. Use according to claim 1 wherein the target site is at least part of the brain, spinal cord and/or spinal nerve.

3. The use according to claim 1 or 2 wherein the use further comprises the step of administration of the vector system to an administration site which is distant from the target site.

4. The use according to claim 3 wherein the administration site is the cerebrospinal fluid.

5. The use according to claim 3 wherein the administration site is a part of the brain.

6. The use according to claim 3 wherein administration is intramuscular or via the peripheral nervous system.

7. The use according to any preceding claim wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine.

8. The use according to any one of claims 1 to 6 wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine and lysine at amino acid 330 is replaced with asparagine, or a variant, fragment or homologue thereof.

9. The use according to any preceding claim wherein the vector system is a viral vector system.

10. The use according to any preceding claim wherein the vector system is a retroviral vector system.

11. The use according any preceding claim, wherein the vector system is used to deliver an EOI.

12. The use according claim 11, wherein the EOI is an NOI.

13. The use according to claim 11 or 12, wherein the EOI is capable of replacing or upregulating the expression of a gene in a target cell.

14. The use according to claim 12 or 13, wherein the EOI is an NOI which is a selection gene, a marker gene or a therapeutic gene.

15. The use according claim 11 or 12, wherein the EOI is capable of blocking or inhibiting the expression of a gene in a target cell.

16. The use according to claim 15 wherein the EOI is antisense RNA, an antibody, an siRNA, a ribozyme, a short hairpin RNA or a microRNA.

17. The use according to any one of claims 11 to 16, wherein the EOI is capable of integrating in the genome of a target cell.

18. The use according to any one of claims 11 to 17, wherein the EOI has a therapeutic effect or codes for a protein that has a therapeutic effect.

19. The use according to any one of claims 11 to 18, wherein the EOI is capable of encoding a protein of interest ("POI").

20. The use according any preceding claim, wherein the vector system is derivable from a lentivirus.

21. The use according to claim 20, wherein the vector system is derivable from EIAV or HIV.

22. A method of treating and/or preventing a disease in a patient comprising administering a vector system as defined in any one of claims 1 to 21 to a patient in need of the same.

23. A pharmaceutical composition comprising a vector system as defined in any one of claims 1 to 22 and a pharmaceutically acceptable carrier, diluent or excipient.

24. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a vector system as defined in any one of claims 1 to 22 to transduce a target cell.

25. A method according to claim 22 or 24, to treat and/or prevent a movement disorder.

26. A method according to claim 25 wherein the movement disorder is Parkinson's disease, a motor neuron disease or Huntington's disease.

27. A method according to claim 22 or 24, to treat and/or prevent a lysosomal storage disease.

28. A method according to claim 27 wherein the lysosomal storage disease is Gaucher disease, Fabry disease, Niemann-Pick disease, gangliosidosis, mucopolysaccharidosis or metachromatic leukodystrophy.

29. A method according to claim 22 or 24, to treat and/or prevent Alzheimer's disease, ALS or pain.

30. A method according to claim 22 or 24 to treat and/or prevent SMA.

31. A method according to claim 22 or 24 to treat and/or prevent an inflammatory neurological disorder.

32. A method according to claim 31 wherein the disorder is an autoimmune neurological disease.

33. A method according to claim 31 or 32 wherein the disorder is Multiple Sclerosis, a stroke, a traumatic brain injury, HIV-related dementia, Alzheimer's disease, prion disease, the Guillian-Barre syndrome, myasthenia gravis, encephalomyelitis, stiff-man syndrome, autoimmune neuritis, motor dysfunction, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy, paraproteinaemic neuropathy, an autoimmune disease of the neuromuscular junction, inflammatory myopathy, autoimmune myositis, a parameoplastic neurological disorder, a neurological complication of connective tissue disease or vasculitis.

34. A method for analysing the effect of a POI in the CNF, comprising the step of using a vector system as defined in any one of the preceding claims.

35. Use of a vector system comprising an EOI to biodistribute the EOI, wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

36. The use according to claim 35 wherein the use further comprises the step of administration of the vector system to an administration site which is distant from the target site.

37. The use according to claim 35 or 36 wherein the vector system is administered intramuscularly, via the cerbrospinal fluid, brain or peripheral nervous system.

38. The use according to any one of claims 35 to 37 wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine.

39. The use according to any one of claims 35 to 37 wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine and lysine at amino acid 330 is replaced with asparagine, or a variant, fragment of homologue thereof.

40. The use according to any one of claims 35 to 39 wherein the vector system is a viral vector system.

41. The use according to any one of claims 35 to 40 wherein the vector system is a retroviral vector system.

42. The use according to any one of claims 35 to 41, wherein the EOI is an NOI.

43. The use according to claim 42, wherein the EOI is capable of replacing or upregulating the expression of a gene in a target cell.

44. The use according to claim 42 or 43, wherein the EOI is an NOI which is a selection gene, a marker gene or a therapeutic gene.

45. The use according to any one of claims 35 to 44, wherein the EOI is capable of blocking or inhibiting the expression of a gene in a target cell.

46. The use according to claim 45 wherein the EOI is antisense RNA, an antibody, an siRNA, a ribozyme, a short hairpin RNA or a microRNA.

47. The use according to any one of claims 35 to 46, wherein the EOI is capable of integrating in the genome of a target cell.

48. The use according to any one of claims 35 to 47, wherein the EOI has a therapeutic effect or codes for a protein that has a therapeutic effect.

49. The use according to any one of claims 35 to 48, wherein the EOI is capable of encoding a protein of interest ("POI").

50. The use according any one of claims 35 to 49, wherein the vector system is derivable from a lentivirus.

51. The use according to claim 50, wherein the vector system is derivable from EIAV or HIV.

52. A method of treating and/or preventing a disease in a patient comprising administering a vector system as defined in any one of claims 35 to 51 to a patient in need of the same.

53. A pharmaceutical composition comprising a vector system as defined in any one of claims 35 to 52 and a pharmaceutically acceptable carrier, diluent or excipient.

54. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a vector system as defined in any one of claims 35 to 52 to transduce a target cell.

55. A method according to claim 52 or 54, to treat and/or prevent a movement disorder.

56. A method according to claim 55 wherein the movement disorder is Parkinson's disease, motor neuron disease or Huntington's disease.

57. A method according to claim 52 or 54, to treat and/or prevent a lysosomal storage disease.

58. A method according to claim 57 wherein the lysosomal storage disease is Gaucher disease, Fabry disease, Niemann-Pick disease, gangliosidosis, mucopolysaccharidosis or metachromatic leukodystrophy.

59. A method according to claim 52 or 54, to treat and/or prevent Alzheimer's disease, ALS or pain.

60. A method according to claim 52 or 54 to treat and/or prevent SMA.

61. A method according to claim 52 or 54 to treat and/or prevent an inflammatory neurological disorder.

62. A method according to claim 61 wherein the disorder is an autoimmune neurological disease.

63. A method according to claim 61 or 62 wherein the disorder is Multiple Sclerosis, a stroke, a traumatic brain injury, HIV-related dementia, Alzheimer's disease, prion disease, the Guillian-Barre syndrome, myasthenia gravis, encephalomyelitis, stiff-man syndrome, autoimmune neuritis, motor dysfunction, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy, paraproteinaemic neuropathy, an autoimmune disease of the neuromuscular junction,

inflammatory myopathy, autoimmune myositis, a parameoplastic neurological disorder, a neurological complication of connective tissue disease or vasculitis.

64. A method for analysing the effect of a POI in the CNS, comprising the step of using a vector system as defined in any one of claims 35 to 63.

65. Use of a vector system to transduce a target site, wherein the vector system travels to the target site by retrograde transport, and wherein the vector system is or comprises at least part of a CVS envelope protein or a mutant, variant, homologue or fragment thereof, and further wherein the target site is at least part of the central nervous system.

66. Use according to claim 65 wherein the target site is at least part of the brain, spinal cord and/or spinal nerve.

67. The use according to claim 58 or 59 wherein the use further comprises the step of administration of the vector system to an administration site which is distant from the target site.

68. The use according to claim 67 wherein the administration site is the cerebrospinal fluid.

69. The use according to claim 67 wherein the administration site is a part of the brain.

70. The use according to claim 67 wherein administration is intramuscular or via the peripheral nervous system.

71. The use according to any one of claims 65 to 70 wherein the vector system is a viral vector system.

72. The use according to claim 71 wherein the vector system is a retroviral vector system.

73. The use according any one of claims 65 to 72, wherein the vector system is used to deliver an EOI.

74. The use according claim 73, wherein the EOI is an NOI.

75. The use according to claim 73 or 74, wherein the EOI is capable of replacing or upregulating the expression of a gene in a target cell.

76. The use according to any one of claims 73 to 75, wherein the EOI is an NOI which is a selection gene, a marker gene or a therapeutic gene.

77. The use according claim 73 or 74, wherein the EOI is capable of blocking or inhibiting the expression of a gene in a target cell.

78. The use according to claim 77 wherein the EOI is antisense RNA, an antibody, an siRNA, a ribozyme, a short hairpin RNA or a microRNA.

79. The use according to any one of claims 73 to 78, wherein the EOI is capable of integrating in the genome of a target cell.

80. The use according to any one of claims 73 to 79, wherein the EOI has a therapeutic effect or codes for a protein that has a therapeutic effect.

81. The use according to any one of claims 73 to 80, wherein the EOI is capable of encoding a protein of interest ("POI").

82. The use according any one of claims 65 to 81, wherein the vector system is derivable from a lentivirus.

83. The use according to claim 82, wherein the vector system is derivable from EIAV or HIV.

84. A method of treating and/or preventing a disease in a patient comprising administering a vector system as defined in any one of claims 65 to 81 to a patient in the need of the same.

85. A pharmaceutical composition comprising a vector system as defined in any one of claims 65 to 84 and a pharmaceutically acceptable carrier, diluent or excipient.

86. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a vector system as defined in any one of claims 65 to 84 to transduce a target cell.

87. A method according to claim 84 or 86, to treat and/or prevent a movement disorder.

88. A method according to claim 87 wherein the movement disorder is Parkinson's disease, a motor neuron disease or Huntington's disease.

89. A method according to claim 84 or 86, to treat and/or prevent a lysosomal storage disease.

90. A method according to claim 89 wherein the lysosomal storage disease is Gaucher disease, Fabry disease, Niemann-Pick disease, gangliosidosis, mucopolysaccharidosis or metachromatic leukodystrophy.

91. A method according to claim 84 or 86, to treat and/or prevent Alzheimer's disease, ALS or pain.

92. A method according to claim 84 or 86 to treat and/or prevent SMA.

93. A method according to claim 84 or 86 to treat and/or prevent an inflammatory neurological disorder.

94. A method according to claim 93 wherein the disorder is an autoimmune neurological disease.

95. A method according to claim 93 or 94 wherein the disorder is Multiple Sclerosis, a stroke, a traumatic brain injury, HIV-related dementia, Alzheimer's disease, prion disease, the Guillian-Barre syndrome, myasthenia gravis, encephalomyelitis, stiff-man syndrome, autoimmune neuritis, motor dysfunction, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy, paraproteinaemic neuropathy, an autoimmune disease of the neuromuscular junction, inflammatory myopathy, autoimmune myositis, a parameoplastic neurological disorder, a neurological complication of connective tissue disease or vasculitis.

96. A method for analysing the effect of a POI in the CNS, comprising the step of using a vector system as defined in any one of claims 65 to 95.

97. Use of a vector system to transduce an *in utero* target site or a target site in a neonate, wherein wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

98. Use according to claim 97 wherein the target site is the at least part of the brain, spinal cord and/or spinal nerve.

99. The use according to claim 97 or 98 wherein the use further comprises the step of administration of the vector system to an administration site which is distant from the target site.

100. The use according to claim 99 wherein the administration site is the cerebrospinal fluid.

101. The use according to claim 99 wherein the administration site is a part of the brain.

102. The use according to claim 99 wherein administration is intramuscular or via the peripheral nervous system.

103. The use according to any one of claims 97 to 101 wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine.

104. The use according to any one of claims 97 to 101 wherein the mutant rabies G envelope protein is a rabies G envelope protein in which arginine at amino acid 333 is replaced with glutamine and lysine at amino acid 330 is replaced with asparagine, or a variant, fragment of homologue thereof.

105. The use according to any one of claims 97 to 104 wherein the vector system is a viral vector system.

106. The use according to claim 105 wherein the vector system is a retroviral vector system.

107. The use according any one of claims 97 to 106, wherein the vector system is used to deliver an EOI.

108. The use according claim 107, wherein the EOI is an NOI.

109. The use according to claim 107 or 108, wherein the EOI is capable of replacing or upregulating the expression of a gene in a target cell.

110. The use according to any one of claims 107 to 109, wherein the EOI is an NOI which is a selection gene, a marker gene or a therapeutic gene.

111. The use according claim 107 or 108, wherein the EOI is capable of blocking or inhibiting the expression of a gene in a target cell.

112. The use according to claim 111 wherein the EOI is antisense RNA, an antibody, an siRNA, a ribozyme, a short hairpin RNA or a microRNA.

113. The use according to any one of claims 107 to 112, wherein the EOI is capable of integrating in the genome of a target cell.

114. The use according to any one of claims 107 to 113, wherein the EOI has a therapeutic effect or codes for a protein that has a therapeutic effect.

115. The use according to any one of claims 107 to 114, wherein the EOI is capable of encoding a protein of interest ("POI").

116. The use according any one of claims 107 to 115, wherein the vector system is derivable from a lentivirus.

117. The use according to claim 116, wherein the vector system is derivable from EIAV or HIV.

118. A method of treating and/or preventing a disease in a patient comprising administering a vector system as defined in any one of claims 97 to 117 to a patient in need of the same.

119. A pharmaceutical composition comprising a vector system as defined in any one of claims 97 to 118 and a pharmaceutically acceptable carrier, diluent or excipient.

120. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a vector system as defined in any one of claims 97 to 118 to transduce a target cell.

121. A method according to claim 118 or 120, to treat and/or prevent a movement disorder.

122. A method according to claim 121 wherein the movement disorder is Parkinson's disease, a motor neuron disease or Huntington's disease.

123. A method according to claim 118 or 120, to treat and/or prevent a lysosomal storage disease.

124. A method according to claim 123 wherein the lysosomal storage disease is Gaucher disease, Fabry disease, Niemann-Pick disease, gangliosidosis, mucopolysaccharidosis or metachromatic leukodystrophy.

125. A method according to claim 118 or 120, to treat and/or prevent Alzheimer's disease, ALS or pain.

126. A method according to claim 118 or 120 to treat and/or prevent SMA.

127. A method according to claim 118 or 120 to treat and/or prevent an inflammatory neurological disorder.

128. A method according to claim 127 wherein the disorder is an autoimmune neurological disease.

129. A method according to claim 127 or 128 wherein the disorder is Multiple Sclerosis, a stroke, a traumatic brain injury, HIV-related dementia, Alzheimer's

disease, prion disease, the Guillian-Barre syndrome, myasthenia gravis, encephalomyelitis, stiff-man syndrome, autoimmune neuritis, motor dysfunction, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy, paraproteinaemic neuropathy, an autoimmune disease of the neuromuscular junction, inflammatory myopathy, autoimmune myositis, a parameoplastic neurological disorder, a neurological complication of connective tissue disease or vasculitis.

130. A method for analysing the effect of a POI in the CNS, comprising the step of using a vector system as defined in any one of claims 97 to 129.

131. An EIAV vector which comprises at least part of a CVS envelope protein or a mutant, variant, homologue or fragment thereof.

132. Use of a vector as defined in claim 131, in the manufacture of a pharmaceutical composition to treat and/or prevent a disease in a subject.

133. A pharmaceutical composition comprising a vector system as defined in claim 131 and a pharmaceutically acceptable carrier, diluent or excipient.

134. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a vector as defined in claim 131 to transduce a target cell.

135. Use of a vector as defined in claim 115 for use as defined in any one of claims 1 to 22, 35 to 52 or 65 to 84 or 97 to 118.

136. A method for analysing the function of a gene, or a protein encoded by a gene, in a target cell, which method comprises the step of inhibiting or blocking the expression of the gene using a vector or vector system as defined in any one of claims 1 to 22, 35 to 52 or 65 to 84, 97 to 118 or 131.

137. A cell transduced with a vector or vector system as defined in any one of 1 to 22, 35 to 52 or 65 to 84, 97 to 118 or 131.

138. An immortalised brain cell, a cell of the spinal cord or motor or sensory neuron cell according to claim 137.

139. Use of an immortalised cell according to claim 137 in the manufacture of a medicament for use in transplantation.

140. A method for treating and/or preventing a disease in a subject in need of same, said method comprising the step of transplanting an immortalised cell according to claim 137 into said subject.

ABSTRACT

VECTOR SYSTEM

Use of a vector system to transduce a target site, wherein the vector system travels to the target site by diffusion, and wherein the vector system is or comprises at least part of a rabies G envelope protein or a mutant, variant, homologue or fragment thereof, or a CVS envelope protein or a mutant, variant, homologue or fragment thereof, and further wherein the target site is at least part of the central nervous system.

Figure 1

SEQ ID NO. 1

NUCLEOTIDE SEQUENCE OF RABIES VIRUS STRAIN ERA

Genbank: locus RAVGPLS, accession M38452

```
   1 aggaaagatg gttcctcagg ctctcctgtt tgtacccctt ctggtttttc cattgtgttt
  61 tgggaaattc cctatttaca cgatactaga caagcttggt ccctggagcc cgattgacat
 121 acatcacctc agctgcccaa acaatttggt agtggaggac gaaggatgca ccaacctgtc
 181 agggttctcc tacatggaac ttaaagttgg atacatctta gccataaaaa tgaacgggtt
 241 cacttgcaca ggcgttgtga cggaggctga aacctacact aacttcgttg gttatgtcac
 301 aaccacgttc aaaagaaagc atttccgccc aacaccagat gcatgtagag ccgcgtacaa
 361 ctggaagatg gccggtgacc ccagatatga agagtctcta cacaatccgt accctgacta
 421 ccgctggctt cgaactgtaa aaaccaccaa ggagtctctc gttatcatat ctccaagtgt
 481 agcagatttg gacccatatg acagatccct tcactcgagg gtcttcccta gcgggaagtg
 541 ctcaggagta gcggtgtctt ctacctactg ctccactaac cacgattaca ccatttggat
 601 gcccgagaat ccgagactag ggatgtcttg tgacattttt accaatagta gagggaagag
 661 agcatccaaa gggagtgaga cttgcggctt tgtagatgaa agaggcctat ataagtcttt
 721 aaaaggagca tgcaaactca agttatgtgg agttctagga cttagactta tggatggaac
 781 atgggtcgcg atgcaaacat caaatgaaac caaatggtgc cctcccgatc agttggtgaa
 841 cctgcacgac tttcgctcag acgaaattga gcaccttgtt gtagaggagt tggtcaggaa
 901 gagagaggag tgtctggatg cactagagtc catcatgaca accaagtcag tgagtttcag
 961 acgtctcagt catttaagaa aacttgtccc tgggtttgga aaagcatata ccatattcaa
1021 caagaccttg atggaagccg atgctcacta caagtcagtc agaacttgga atgagatcct
1081 cccttcaaaa gggtgtttaa gagttggggg gaggtgtcat cctcatgtga acggggtgtt
1141 tttcaatggt ataatattag gacctgacgg caatgtctta atcccagaga tgcaatcatc
1201 cctcctccag caacatatgg agttgttgga atcctcggtt atccccccttg tgcaccccct
1261 ggcagacccg tctaccgttt tcaaggacgg tgacgaggct gaggattttg ttgaagttca
1321 ccttcccgat gtgcacaatc aggtctcagg agttgacttg ggtctcccga actgggggaa
1381 gtatgtatta ctgagtgcag gggccctgac tgccttgatg ttgataattt tcctgatgac
1441 atgttgtaga agagtcaatc gatcagaacc tacgcaacac aatctcagag ggacagggag
1501 ggaggtgtca gtcactcccc aaagcgggaa gatcatatct tcatgggaat cacacaagag
1561 tgggggtgag accagactgt gaggactggc cgtcctttca acgatccaag tcctgaagat
1621 cacctcccct tgggggttc tttttaaaaa
```

Figure 2

SEQ ID NO. 2

AMINO ACID SEQUENCE OF RABIES VIRUS STRAIN ERA

Genbank: locus RAVGPLS, accession M38452
PID:g333574"

MVPQALLFVPLLVFPLCFGKFPIYTILDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSG
FSYMELKVGYILAIKMNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYN
WKMAGDPRYEESLHNPYPDYRWLRTVKTTKESLVIISPSVADLDPYDRSLHSRVFPSGK
CSGVAVSSTYCSTNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYK
SLKGACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPPDQLVNLHDFRSDEIEHLVVEEL
VRKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTW
NEILPSKGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHMELLESSVIP
LVHPLADPSTVFKDGDEAEDFVEVHLPDVHNQVSGVDLGLPNWGKYVLLSAGALTALML
IIFLMTCCRRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGETRL

Figure 3

SEQ ID NO:3 ERAdm

ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGTTTTGGGAA
ATTCCCTATTTACACGATCCCAGACAAGCTTGGTCCCTGGAGCCCGATTGACATACATC
ACCTCAGCTGCCCAAACAATTTGGTAGTGGAGGACGAAGGATGCACCAACCTGTCAGGG
TTCTCCTACATGGAACTTAAAGTTGGATACATCTTAGCCATAAAAATGAACGGGTTCAC
TTGCACAGGCGTTGTGACGGAGGCTGAAACCTACACTAACTTCGTTGGTTATGTCACAA
CCACGTTCAAAAGAAAGCATTTCCGCCCAACACCAGATGCATGTAGAGCCGCGTACAAC
TGGAAGATGGCCGGTGACCCCAGATATGAAGAGTCTCTACACAATCCGTACCCTGACTA
CCGCTGGCTTCGAACTGTAAAAACCACCAAGGAGTCTCTCGTTATCATATCTCCAAGTG
TAGCAGATTTGGACCCATATGACAGATCCCTTCACTCGAGGGTCTTCCCTAGCGGGAAG
TGCTCAGGAGTAGCGGTGTCTTCTACCTACTGCTCCACTAACCACGATTACACCATTTG
GATGCCCGAGAATCCGAGACTAGGGATGTCTTGTGACATTTTTACCAATAGTAGAGGGA
AGAGAGCATCCAAAGGGAGTGAGACTTGCGGCTTTGTAGATGAAAGAGGCCTATATAAG
TCTTTAAAAGGAGCATGCAAACTCAAGTTATGTGGAGTTCTAGGACTTAGACTTATGGA
TGGAACATGGGTCGCGATGCAAACATCAAATGAAACCAAATGGTGCCCTCCCGATCAGT
TGGTGAACCTGCACGACTTTCGCTCAGACGAAATTGAGCACCTTGTTGTAGAGGAGTTG
GTCAGGAAGAGAGAGGAGTGTCTGGATGCACTAGAGTCCATCATGACAACCAAGTCAGT
GAGTTTCAGACGTCTCAGTCATTTAAGAAAACTTGTCCCTGGGTTTGGAAAAGCATATA
CCATATTCAACAAGACCTTGATGGAAGCCGATGCTCACTACAACTCAGTCATGACTTGG
AATGAGATCCTCCCCTCAAAAGGGTGTTTAAGAGTTGGGGGGAGGTGTCATCCTCATGT
GAACGGGGTGTTTTTCAATGGTATAATATTAGGACCTGACGGCAATGTCTTAATCCCAG
AGATGCAATCATCCCTCCTCCAGCAACATATGGAGTTGTTGGAATCCTCGGTTATCCCC
CTTGTGCACCCCCTGGCAGACCCGTCTACCGTTTTCAAGGACGGTGACGAGGCTGAGGA
TTTTGTTGAAGTTCACCTTCCCGATGTGCACAATCAGGTCTCAGGAGTTGACTTGGGTC
TCCCGAACTGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGTTG
ATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACGCAACACAA
TCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGCGGGAAGATCATATCTT
CATGGGAATCACACAAGAGTGGGGGTGAGACCAGACTGTGA

Figure 4

SEQ ID NO:4 CVS rabies virus glycoprotein

ATGGTTCCTCAGGTTCTTTTGTTTGTACTCCTTCTGGGTTTTTCGTTGTGTTTCGGGAA
GTTCCCCATTTACACGATACCAGACAAACTTGGTCCCTGGAGCCCTATTGACATACACC
ATCTCCGCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGATGTATCAACCTGTCCGGG
TTCTCCTACATGGAACTCAAAGTGGGATACATCTCAGCCATCAAAGTGAACGGGTTCAC
TTGCACAGGTGTTGTGACAGAGGCAGAGACCTACACCAACTTTGTTGGTTATGTCACAA
CCACATTCAAGAGAAAGCATTTCCGCCCCACCCCAGACGCATGTAGAGCCGCGTATAAC
TGGAAGATGGCCGGTGACCCCAGATATGAAGAGTCCCTACAAAATCCATACCCCGACTA
CCACTGGCTTCGAACTGTAAGAACCACCAAAGAGTCCCTCATTATCATATCCCCAAGTG
TGACAGATTTGGACCCATATGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCGGAAAG
TGCTCAGGAATAACGGTGTCCTCTACCTACTGCTCAACTAACCATGATTACACCATTTG
GATGCCCGAGAATCCGAGACCAGGGACACCTTGTGACATTTTTACCAATAGCAGAGGGA
AGAGAGCATCCAACGGGAACAAGACTTGCGGCTTTGTGGATGAAAGAGGCCTGTATAAG
TCTCTAAAAGGAGCATGCAGGCTCAAGTTATGTGGAGTTCTTGGACTTAGACTTATGGA
TGGAACATGGGTCGCGATGCAAACATCAGATGAGACCAAATGGTGCTCTCCAGATCAGT
TGGTGAATTTGCACGACTTTCGCTCAGACGAGATTGAGCATCTCGTTGTGGAGGAGTTA
GTCAAGAAAAGAGAGGAATGTCTGGATACATTAGAGTCCATCATGACCACCAAGTCAGT
AAGTTTCAGACGTCTCAGTCACCTGAGAAAACTTGTCCCAGGGTTTGGAAAAGCATATA
CCATATTCAACAAAACCTTGATGGAGGCTGATGCTCACTACAAGTCAGTCCGGACCTGG
AATGAGATCATCCCCTCAAAAGGGTGTTTGAAAGTTGGAGGAAGGTGCCATCCTCATGT
GAACGGGGTGTTTTTCAATGGTATAATATTAGGGCCTGACGACCGTGTCCTAATCCCAG
AGATGCAATCATCCCTCCTCCGGCAACATATGGAGTTGTTGGAATCTTCAGTTATCCCC
CTGATGCACCCCCTGGCTGACCCTTCTACAGTTTTCAAAGAAGGTGATGAGGCTGAGGA
TTTTGTTGAAGTTCACCTCCCCGATGTGTACAAACAGATCTCAGGGGTTGACCTGGGTC
TCCCGAACTGGGGAAAGTATGTATTGATGACTGCAGGGGCCATGATTGGCCTGGTGTTG
ATATTTTCCCTAATGACATGGTGCAGAAGAGCCAATCGACCAGAATCGAAACAACGCAG
TTTTGGAGGGACAGGGGGGAATGTGTCAGTCACTTCCCAAAGCGGAAAAGTCATACCTT
CATGGGAATCATATAAGAGTGGAGGTGAGATCAGACTGTGA

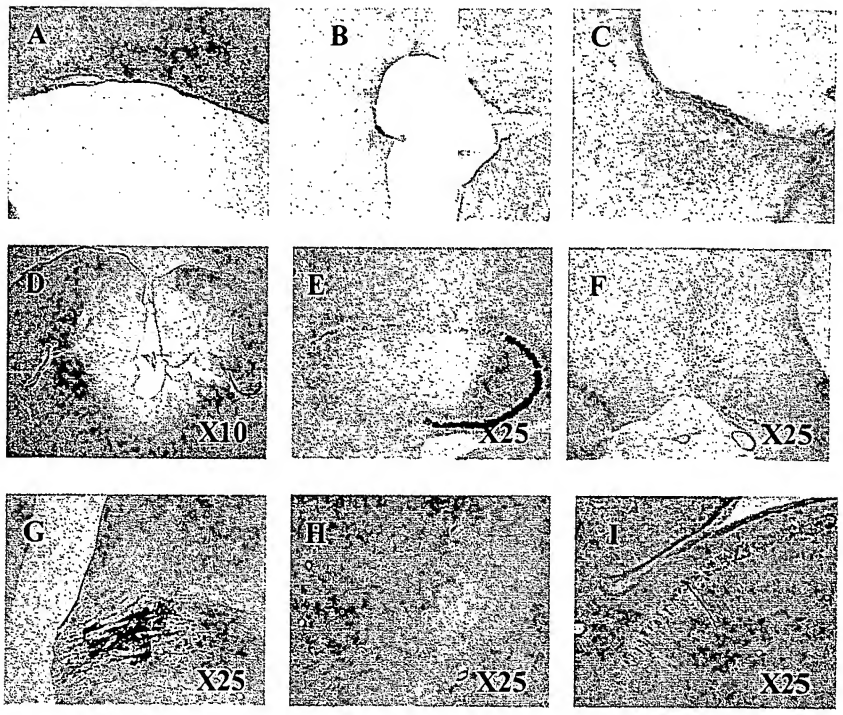


Figure 5

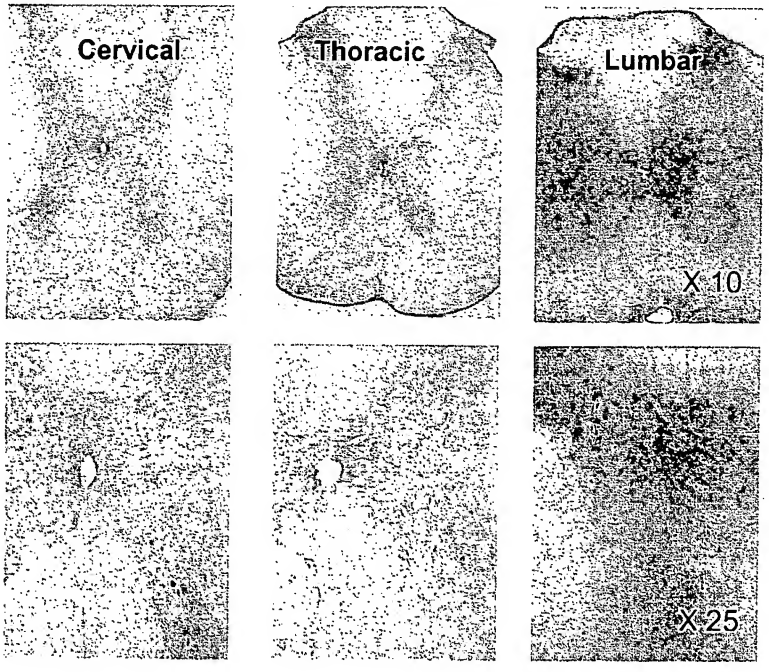
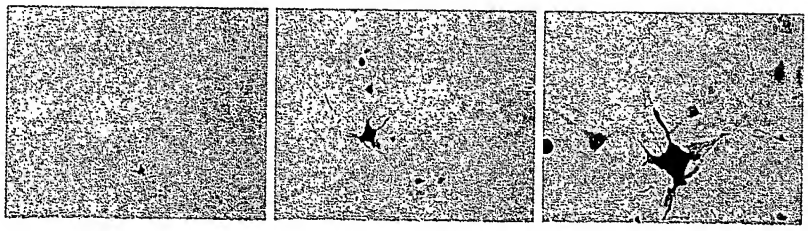


Figure 6

Figure 7

x-gal staining : spinal cord



β-gal immunostaining : spinal cord

x-gal staining : gastrocnemius muscle

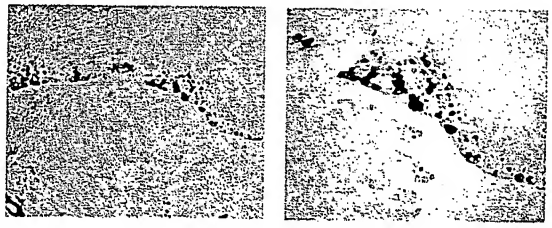


Figure 8

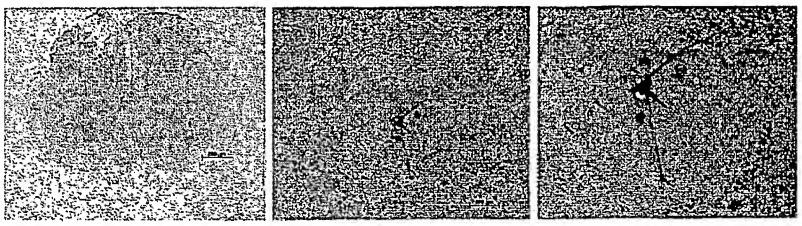
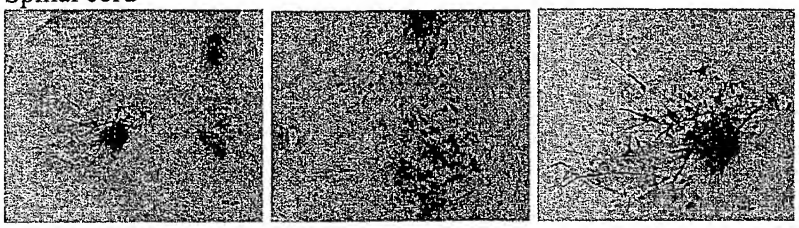
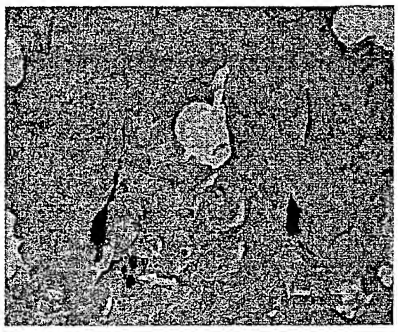


Figure 9

Spinal cord



Cortex



Brain stem

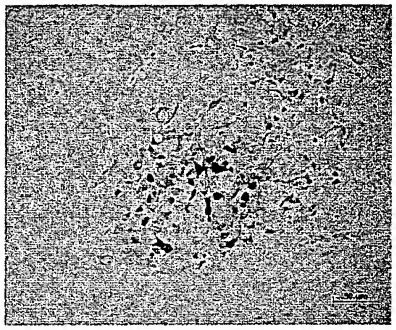
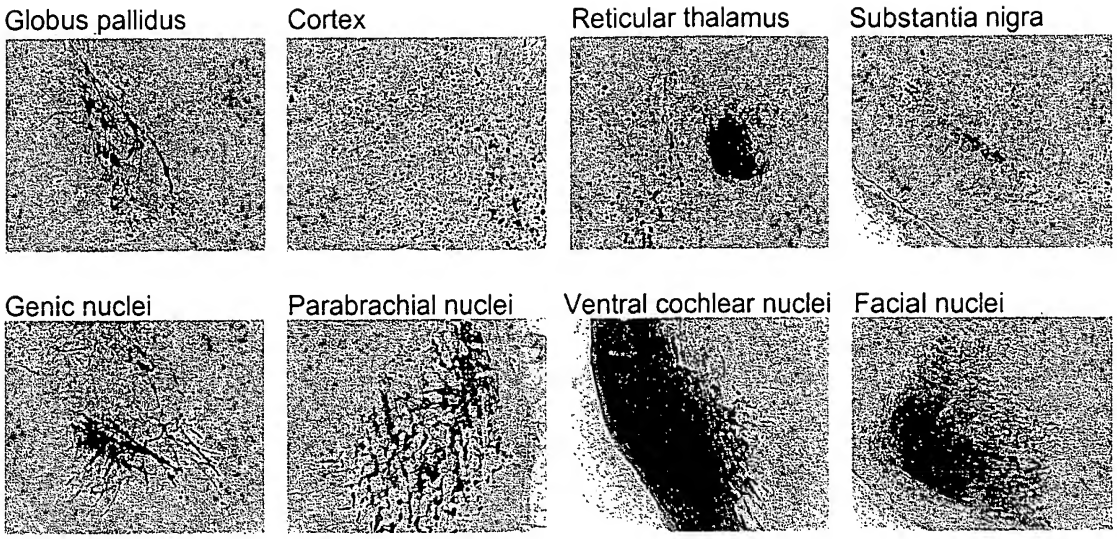


Figure 10


Globus pallidus
Cortex
Reticular thalamus
Substantia nigra
Genic nuclei
Parabrachial nuclei
Ventral cochlear nuclei
Facial nuclei